ECOLOGICAL DRIVERS OF MERCURY ACCUMULATION IN THREESPINE STICKLEBACK FISH

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Abstract

I utilized the ecological diversity displayed in the Cook Inlet adaptive radiation of threespine stickleback (*Gasterosteus aculeatus* species complex, hereafter 'stickleback') to examine the drivers of intra- and inter-population variation in total mercury (THg) concentrations. I examined the importance of sex, trophic position (TP), and habitat-specific foraging (measured as the proportion of the diet derived from benthic sources; α) in stickleback from Benka Lake, Alaska, a lake with both benthic and limnetic ecotypes. The results demonstrate that both sex and habitat-specific foraging are important determinants of THg concentrations in this threespine stickleback population. Specifically, male stickleback and stickleback foraging in limnetic habitats had higher THg concentrations than females or benthic foraging individuals. Further, I found that the relationships between THg concentration, TP, and α differed between the sexes such that TP and α were of approximately equal importance in female fish but TP was more important than α in male fish.

I assessed the relative importance of these same factors in determining THg concentrations of stickleback from six lakes spanning a range of trophic ecologies. Across populations, I found sex and TP to be more important determinants of THg concentrations than reliance on benthic prey; however, there was substantial variation in the relative importance of these parameters in individual lakes. Across lakes I also found a positive correlation between THg concentrations in stickleback and the reliance on benthic prey, and my data suggest that differences in the bioavailability of Hg in the lakes were responsible for this relationship.

I investigated temporal variation in the THg concentrations of Benka Lake stickleback. The temporal patterns observed in stickleback likely result from numerous physiological and ecological processes. I found that the importance and magnitude of these factors acting upon THg concentrations varied between sexes, ecotypes, or both, though the directions of the relationships were consistent across groups. Despite this variation, TP was consistently the most important determinant of Hg concentrations.

Collectively, the results of this dissertation demonstrate that the ecological factors driving THg concentrations in stickleback are complex, likely integrate multiple confounding interactions, and often vary by sex, ecotype, and population (lake). To improve our understanding of the mechanisms underlying Hg bioaccumulation, future research should utilize experimental studies and larger numbers of wild populations to examine the independent effects of these variables within the context of varying physiologies and ecologies.

Dedication

To Jim and Ron Willacker for all they have taught me,

their unwavering support, and constant encouragement.

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The chapters comprising this dissertation were written by me, James Willacker; however, the completion of this research would not have been possible without the contributions of my co-authors. Authorship on the manuscripts is based on intellectual contributions to the work. Frank von Hippel and Todd O'Hara assisted with the design, implementation, and editing of all three chapters. Collin Eagles-Smith contributed to the study design and particularly the statistical approaches, conducted many of the mercury analyses presented, and provided editorial feedback for Chapters 3 and 4. Kerri Ackerly contributed thoughtful feedback on study design in addition to conducting much of the collecting and preparation of samples for Chapter 2.

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Chapter 1: General Introduction

1.1 Introduction to Mercury

The element mercury (Hg), although naturally occurring and widespread, has no known biological function and in fact can result in potentially severe health hazards to biota [1]. However, mercury's many unique properties, particularly it being the only metal that remains fluid at room temperature and its ready formation of amalgams with other metals, has resulted in a long history of extraction for human activities. Compounding these intentional extractions, unintentional release of Hg due to anthropogenic activities, particularly the combustion of Hg rich fossil fuels, has resulted in substantial alterations to the global mercury cycle. Thus, while natural emissions of Hg via geological processes such as volcanism and the weathering of Hg rich rocks has previously been balanced by the long-term sequestration of Hg into deep ocean sediments, modern human activities have increased emission rates such that they outpace depositional rates [2]. Despite these changes, nearly 99% of global Hg is estimated to remain buried in marine sediments [3].

Mercury occurs in three oxidation states; elemental or metallic Hg (Hg^0), the mercurous ion (Hg^+) also referred to as monovalent Hg, and the mercuric ion (Hg^{2+}) also referred to as divalent Hg. The latter two forms can form a variety of inorganic and organic compounds (collectively these forms are hereafter referred to as either inorganic or organic Hg), and the differences between these compounds are critical in determining the distribution, fate, and toxicology of Hg in the environment.

Inorganic Hg comprises the bulk of both natural and anthropogenic emissions [4], is capable of being transported and deposited over great distances [5, 6], and is often the primary form of mercury present in ecosystems [7]. Despite these characteristics, inorganic Hg is retained by biota to a lesser extent than organic forms, and thus accumulates in biota to a lesser extent, than organic Hg [8]. However, inorganic Hg is readily converted to more biologically active organic Hg in a variety of aquatic habitats and biota [2, 9, 10]. Thus, the overall risk posed by Hg is determined not only by Hg inputs into a system, but also by transformation of Hg between different forms and the route of exposure.

Of the various forms of Hg, the organic compounds incorporating monomethylmercury (MeHg+) are the most prevalent in freshwater biota and of the greatest toxicological concern. Monomethylmercury is known to bioaccumulate (i.e., buildup in the tissues of biota) and biomagnify (i.e., increase in concentration from prey to consumer) in aquatic food webs such that some consumers can have concentrations in their tissue many times the threshold for toxic effects. The bioaccumulation and biomagnification properties of MeHg+ in biota are due largely to the high affinity between MeHg+ and the sulfhydrl (thiol) group of proteins, which results in the sequestration of MeHg+ in tissues, thus hindering elimination. Because MeHg+ is bound strongly to proteins, it is more efficiently transferred from prey to consumer than inorganic forms [11]. Therefore, both the absolute amount of Hg and the proportion of total Hg (THg) as MeHg+ typically increase with increasing trophic position [12, 13]. Further, the affinity between MeHg+ and proteins often (though not always) results in

MeHg+ comprising nearly all Hg in biota [11, 14] despite MeHg+ often making up a small proportion of the THg in an ecosystem [7], accounting for the fact that nearly all Hg in consumers results from dietary exposure [15, 16].

The concerns regarding the prevalence of MeHg+ are due to its severe toxicological effects on both wildlife and humans. All methylmercuries are potent neurotoxins; dimethylmercury, which is abundant in some marine systems, is particularly toxic though MeHg+ is more widespread in freshwater systems and nearly as toxic [1, 17]. Monomethylmercury impacts numerous developmental processes, particularly neurological development, but also the development of muscular, cardiovascular, renal, and endocrine features. While the impacts of MeHg+ are most dramatic in young individuals undergoing development, high MeHg+ exposure can also result in neurological pathology in adults. This pathology is often manifested as sensory deterioration, loss of equilibrium, irrational behavior or impairment of muscular control [17]. These symptoms occur at levels observed in the environment, and result in reduced foraging efficiency, survival or reproductive success of fish and wildlife [18-20].

The prevalence and toxicity of MeHg+ make it a major risk to both wildlife and humans. In the United States, MeHg+ contamination contributes to nearly 80% of all fish consumption advisories with many states issuing statewide restrictions on fish consumption [21]. Because of these concerns, Hg is one of the most widely studied environmental contaminants. However, the Hg biogeochemical cycle is exceedingly complex and despite the extensive literature on Hg in the environment, there are still many aspects of Hg ecodynamics that are poorly understood. In particular, the processes

regulating Hg accumulation in fishes are of special interest since fish consumption is the major exposure route for most people and wildlife [15, 22-24].

1.2 Mercury Bioavailability

Mercury concentrations in fish are often variable among waterbodies. These differences occur even when waterbodies are adjacent and seemingly receive similar inputs of Hg [25-27] suggesting that these differences arise primarily from differential processing of Hg in the ecosystems themselves. In particular, differences in the production and degradation of MeHg+ play a major role in determining the potential for bioaccumulation within a system.

As previously mentioned, nearly all atmospherically deposited Hg, which represents the primary input of Hg to many lakes in some regions [6], is in the form of inorganic Hg^{2+}. Thus, in order to become MeHg+ much of the deposited Hg must first be transformed from inorganic forms. This transformation (methylation) is mediated by sulfur or iron reducing bacteria at the oxic-anoxic interface of sediments, and to a lesser degree open waters, of aquatic environments. By regulating the availability of MeHg+ in the environment, relative methylation and de-methylation rates play critical roles in determining the effective pathways of Hg within the system [10, 28-30]. Thus, lake and watershed characteristics that enhance Hg methylation rates are widely implicated in the elevation of Hg concentrations in biota [29, 31, 32]. These characteristics include the presence of wetlands, which provide highly productive sites for methylation [32], the availability of high quality organic matter and particularly sulfate substrates which

stimulate productivity of the methylating microbial communities [31], and lower pH which reduces the ability of organic matter in the sediments to sequester MeHg+ [33].

Despite the importance of the methylation and demethylation processes, the bioavailability of Hg is also impacted by other factors. For example, high dissolved organic carbon (DOC) concentrations result in the binding of available MeHg+ and can facilitate its removal from a system via down-stream transport [34-36]. Alternatively, sorption of MeHg+ to suspended particulate matter can result in higher MeHg+ ingestion rates by lower trophic level consumers and thus enhanced uptake at the base of the food web [29, 37].

1.3 Trophic Dynamics of Mercury

Since MeHg+ in biota is primarily the result of dietary exposure, uptake at the base of the food web has a strong impact on the overall accumulation of MeHg+ [13, 29]. The bioconcentration (i.e., increased concentration of Hg in tissues compared to the surrounding water) of Hg by pelagic phytoplankton is of particular importance since this process can result in Hg concentrations several thousand to millions of times greater than the surrounding water [13, 29, 38, 39]. In contrast, bioconcentration factors in benthic algae are much lower [40]. Thus, MeHg+ concentrations in primary producers integrate the many effects of Hg inputs, methylation and demethylation rates, chemical sequestration of available MeHg+, and differential uptake into the food web.

Once it has entered the food web, the bioaccumulation and biomagnification of MeHg+ can be further altered by the characteristics of the food web. While high

ecosystem productivity can increase the production of MeHg+ in sediments [31], it can also lead to “bloom dilution”, which is the distribution of available MeHg+ among a larger number of organisms and thus the ultimate reduction in the Hg concentrations in individuals [41, 42]. The length and breadth of food webs also play important roles in determining Hg concentrations in top consumers. For example, longer food webs (i.e., those with more trophic interactions) result in higher Hg concentrations in top consumers even when concentrations at the base of the food web are similar and the consumers involved are the same [43].

In lakes with distinct littoral (referred to as benthic in some literature) and limnetic (pelagic) habitats, differential use of these environments can also alter exposure to Hg in consumers [44-47]. However, the role of habitat-specific foraging in determining Hg concentrations in consumers is not consistent across studies and higher Hg concentrations have been measured in both benthic and limnetic consumers. These inconsistencies may arise from differences in Hg bioavailability and concentrations at the bases of benthic and limnetic food webs, differences in the lengths of the food webs, or due to differential transfer of Hg through the food webs [12].

Both the length and breadth of food webs often differ based on their community composition and these differences are also known to alter Hg dynamics. For example, Cabana et al. [43] demonstrated that the presence of rainbow smelt (*Osmerus mordax*) in lake food webs significantly increased the accumulation of Hg in lake trout (*Salvelinus namaycush*) by increasing both the length of the food web and the proportion of lake trout diet derived from pelagic sources. Similarly, Eagles-Smith et al. [48] demonstrated

that the invasion of a planktivorus forage fish resulted in dramatic increases in the Hg concentrations of other fish species that were forced to consume more contaminated benthic prey items. These studies suggest a need to account for such inter-specific interactions when examining Hg bioaccumulation in aquatic food webs.

1.4 Characteristics of Individuals that Influence Mercury Dynamics

In addition to the myriad ways in which ecosystem characteristics alter Hg dynamics, the characteristics of the individuals composing populations also influence Hg levels in biota. In particular, dietary specialization, age, size, growth, and sex result in differential accumulation of Hg in some fishes.

The role of trophic position and habitat-specific foraging have been discussed above in the context of their roles at the lake level, but diet is ultimately a trait of individuals and as such has a direct impact on Hg concentrations of individual consumers. Thus, in populations composed of individuals specializing on different portions of a food web, dietary variation can play a major role in determining variation in the Hg concentration of individuals [12, 44, 46, 47, 49].

Because MeHg+ is assimilated more rapidly than it is eliminated, the net accumulation of Hg is positively correlated with age [36]. Thus, older individuals or longer-lived species typically accumulate Hg to much higher levels than those of young/short-lived individuals. This pattern is exacerbated by the fact that longer-lived individuals also tend to occupy higher trophic positions in aquatic food webs, thus suffering the effects of increased bioaccumulation and biomagnification [50]. The effects

of body size on Hg accumulation are closely linked to those of age since older fish are typically larger as well. However, bioenergetics modeling suggests that the elimination of Hg is also reduced in larger individuals irrespective of age [51].

Growth rates, or more specifically growth efficiencies (i.e., the amount of mass accumulated by a consumer per unit mass consumed), are also important determinants of Hg bioaccumulation in fishes [52]. Growth rates and Hg concentrations are often negatively correlated due to the effects of "growth dilution"; a process analogous to the "bloom dilution" discussed previously [42]. Thus, slower growing individuals, such as poor competitors, residents of low productivity systems, stressed or older individuals and those with very high energetic demands display higher Hg concentrations than faster growing individuals [12, 52].

In many species of fish Hg concentrations differ between females and males, with females typically, though not always [53, 54], having lower Hg concentrations than males [47, 55-63]. This difference has been attributed to losses of Hg in eggs [55, 56], though there is little evidence for this because eggs typically have low Hg compared to other tissues [58-60]. The differences between males and females have also be ascribed to differences in the foraging ecologies of the sexes [47, 63] and differences in the growth dynamics of females and males [54, 60-63].

1.5 The Threespine Stickleback Model

The threespine stickleback (*Gasterosteus aculeatus* species complex; hereafter referred to as 'stickleback') is a small fish that inhabits marine, estuarine, and freshwater

habitats throughout the Holarctic region. Freshwater stickleback display phenomenal phenotypic diversity in part due to their expansive geographical range and the variety of habitats in which they are found, from tiny ephemeral streams in arid desert regions to large Arctic lakes [64]. In contrast, marine stickleback, while also widely distributed and the ultimate ancestor of all freshwater populations, display remarkable phenotypic conformity across their range and over millions of years [65].

The phenotypic diversity of freshwater stickleback is particularly notable due to the high incidence of repeated parallel evolution between populations [65, 66]. Stickleback across their range have repeatedly evolved distinct trophic ecotypes specializing on the utilization of either near shore littoral habitats and macroinvertebrate prey (benthic ecotypes) or open water limnetic habitats and zooplankton prey (limnetic ecotypes). Benthic and limnetic ecotypes are relatively common and widespread in freshwater fishes, occurring not only in stickleback but also in sunfishes (Centrarchidae), smelts (Osmeridae), whitefish (Coerigonidae), charr (*Salvinus* spp.), and various other salmonids (Salmonidae) [67-70].

In stickleback these ecotypes have evolved both allopatrically in thousands of lakes, and sympatrically in several lakes along the west coast of North America [71]. Many aspects of stickleback biology are linked to foraging [65, 72], and these links have been intensively studied in the sympatric species pairs of British Columbia [73-78] and a few well known allopatric populations in the Cook Inlet Basin of Alaska [64, 72, 79-82].

The morphological differences between these two ecotypes consist of numerous traits that influence the foraging efficiency of fish feeding on their respective types of

prey [64, 82]. Benthic fish have deeper heads and bodies, fewer more widely spaced gill rakers, more robust cranial morphology, and smaller eyes, while limnetic ecotypes have traits that are well suited for feeding on planktonic prey including more fusiform body shape, a longer narrower snout, numerous fine gill rakers, and relatively large eyes [64]. These morphological differences are correlated with several ecological traits including diet, trophic position, and habitat use [64, 83, 84]. Additionally, benthic and limnetic ecotypes differ in several key reproductive characteristics including allocations to reproductive tissues, behavior, and nesting sites [65, 72], thus providing the opportunity for reproductive isolation between the two ecotypes where they occur together. In laboratory common-garden experiments the major differences between benthic and limnetic stickleback are maintained and hybrids are intermediate, indicating that these traits are heritable [65, 84]. Further, there is differential fitness of the ecotypes in their respective habitats [76, 84], indicating that the distinction between ecotypes is ecologically and evolutionarily relevant.

The stickleback species complex provides a powerful model for studying Hg dynamics. In particular, the ecological diversity demonstrated both within and between lakes provides a valuable opportunity to examine the relative importance of individual variation in comparison to lake and watershed scale variables in determining Hg concentrations of fishes. The rich history of stickleback as a model in the fields of behavior, ecology, genetics, and ecological speciation has resulted in the ecological characterization of thousands of populations around the globe, providing an invaluable foundation on which investigations of contaminant ecodynamics can be based [65, 85,

86]. Further, many of these populations are independently derived but ecologically convergent, allowing for replication of ecological conditions and thus increasing the power of such investigations. These characteristics coupled with their wide geographic range and distribution in a variety of habitats makes stickleback a prime candidate for environmental monitoring.

1.6 General Objectives

Few of the factors influencing Hg bioaccumulation act independently. Complex biological and chemical reactions occurring at individual, population, community, ecosystem, and landscape scales interact to determine patterns of Hg bioaccumulation and the ultimate result is not always predictable [87]. In order to understand these interactions and ultimately the drivers of Hg bioaccumulation in fishes, it is critical that we first understand individual factors. This dissertation addresses some of these factors by examining the roles of ecological characteristics (trophic position, habitat-specific foraging, and Hg bioavailability to primary consumers) and features of individuals (sex, size, and body condition) in determining patterns of Hg bioaccumulation in threespine stickleback from the Cook Inlet Basin of Alaska.

In chapter 2 (cited throughout the dissertation as Willacker et al. 2013 [47]), I utilized a naturally polymorphic population of stickleback from Benka Lake, Alaska to understand the role of habitat specific foraging in determining Hg concentrations of stickleback. I measured THg and stable isotope ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) in reproductive (age 2) females and males of both benthic and limnetic ecotypes.

Specifically, stable isotope data were used to estimate trophic position and percent benthic carbon utilized (α). Levels of THg, trophic position and α were used to address whether THg concentrations varied between ecotypes and sexes and to investigate the roles of trophic position and α in determining sex and ecotype differences. This study provides a means to examine the relative importance of α and trophic position in the accumulation of THg in a single population, and establishes the foundation for future studies examining the relative importance of these factors in comparison to inter-population variation.

In chapter 3, I evaluated THg concentrations in stickleback from Cook Inlet populations (i.e., groups of potentially interbreeding individuals; hereafter synonymous with lakes) spanning a range of trophic ecologies – including both benthic and limnetic extreme ecotypes as well as populations characterized by more intermediate ecologies – in order to determine whether the factors examined in chapter 2 play similar roles in determining inter-lake Hg concentrations. I used stable isotopes of carbon and nitrogen as well as stomach content data to determine trophic position and α, and then related these data to variation in THg concentrations both within and between populations. Additionally, I assessed whether these relationships varied between sexes both within and among populations. Finally, I related variation in the THg concentration in stickleback to variation in Hg bioavailability at the base of each food web. These data provide a means of assessing the relative importance of trophic factors in determining inter-population variation in Hg accumulation.

In chapter 4, I again examined Hg bioaccumulation in Benka Lake stickleback, this time with the aim of understanding the factors underlying temporal variation in Hg accumulation and the differences between females and males. To this end I measured THg concentrations in female and male stickleback each week over the course of a 12 week summer breeding season and coupled these data to concurrent measurements of trophic position, habitat use, relative condition and size of individual fish in order to identify the relative importance of each factor over time. Together these data allow for the isolation of temporal variability due to an individual's ecological and physical characteristics and thus provide insights into the processes underlying Hg dynamics.

Collectively, these three studies address important gaps in our understanding of Hg bioaccumulation in fishes and provide insights into the complexities inherent in the ecodynamics of Hg. As such, they provide a valuable foundation on which processes occurring at community, ecosystem, and landscape scales can be examined.

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Chapter 2: Habitat-specific foraging and sex determine mercury concentrations in sympatric benthic and limnetic ecotypes of threespine stickleback[1]

2.1 Abstract

Mercury (Hg) is a widespread environmental contaminant known for the neurotoxicity of the methylated forms, especially monomethyl mercury, which bioaccumulates and biomagnifies in aquatic food webs. Mercury bioaccumulation and biomagnification rates are known to vary among species utilizing different food webs (benthic versus limnetic) within and between systems. I assessed if carbon and nitrogen stable isotope values and total Hg (THg) concentrations differed between sympatric benthic and limnetic ecotypes and sexes of threespine stickleback fish (*Gasterosteus aculeatus*) from Benka Lake, Alaska (USA). The mean THg concentration in the limnetic ecotype was significantly higher (26 ng/g dw; 16.1%) than that of the benthic ecotype. Trophic position and percent benthic carbon utilized were both important determinants of THg concentration; however, in females the two variables were of approximately equal importance whereas in males trophic position clearly explained more of the variance than percent benthic carbon. Additionally, strong sex effects (45 ng/g dw; 29.4%) were observed in both ecotypes with female fish having lower THg concentrations than males.

[1] Willacker, J. J, F. A. von Hippel, K. L. Ackerly, and T. M. O'Hara. 2013. Habitat-specific foraging and sex determine mercury concentrations in sympatric benthic and limnetic ecotypes of threespine stickleback. *Environmental Toxicology and Chemistry* 32: 1623-1630.

These results indicate that trophic ecology and sex are both important determinants of Hg contamination even within a single species and lake, and likely play a role in governing Hg concentrations in higher trophic levels.

2.2 Keywords

Benthic carbon, Bioaccumulation, Biomagnification, *Gasterosteus aculeatus*, Trophic position

2.3 Introduction

Considerable variation has been observed in the bioaccumulation and biomagnification of Hg in fishes from different systems. This variation has been attributed variously to differences in lake and watershed level factors, the biology of individuals, and the structure and function of food webs. Lake and watershed-dependent factors such as watershed morphology, lake chemistry, and proximity to Hg sources affect Hg methylation and demethylation reactions and thus determine the bioavailability of Hg in aquatic systems [1,2]. Differences in the age, growth rate, body condition and sex of individuals composing a population also influence Hg accumulation in various compartments of a system. In many systems older animals exhibit higher concentrations and body burdens [3], and the concentrations of Hg in tissues are often influenced by the composition of the tissue and the process of growth dilution [4]. Further, females often have lower concentrations of Hg in their tissues, though it is not widely agreed what mechanism is responsible [5-9].

The structure of food webs largely establishes the pathways for bioavailable Hg through aquatic systems [10, 11]. While both trophic position (TP; i.e., the vertical position of an individual within a food web [12, 13]) and underlying dietary pathways of consumers (i.e., 'horizontal' position in a food web, such as pelagic vs. benthic feeders [11, 14]) influence the concentration and biomagnification of contaminants in aquatic ecosystems [10, 15], these factors may or may not act independently [11]. For example, Kidd et al. [16] found that in Lake Malawi, concentrations of Hg were higher in biota connected to pelagic food webs than in species of a similar TP relying on benthic food sources, despite similar biomagnification rates in the two habitats.

While the importance of TP and foraging habitat have been used to explain differences in contaminant concentrations between species in the same system [16, 17-22] and between populations in separate systems [1, 2, 23, 24], the possibility of the same factors explaining intra-population differences has only recently received attention. Many species of freshwater fish display ecological divergence between individuals feeding on macroinvertebrates associated with littoral habitats (benthic ecotypes) and individuals feeding on zooplankton in the limnetic zone (limnetic ecotypes) [25]. Though these individuals of the same species inhabit the same system, they utilize largely separate food webs and likely have different pathways of dietary contaminant exposure.

The threespine stickleback (*Gasterosteus aculeatus*, hereafter stickleback) fish model provides an excellent system for examining the role of habitat specific foraging in determining Hg concentrations. The stickleback is a widespread generalist consumer found throughout much of the northern hemisphere [26]. In Alaska, stickleback are the

primary (sometimes the only) fish in many lakes, and are the principal prey of many sport-fish and bird species [26]. Stickleback display differentiation between benthic and limnetic ecotypes both allopatrically in thousands of lakes around the northern hemisphere, and sympatrically in several lakes along the Pacific coast of North America [25-27]. Sympatric ecotypes of stickleback provide an opportunity to examine the effects of habitat specific foraging on Hg concentrations in a single species of fish from the same lake.

Benthic and limnetic ecotypes of stickleback have been extensively studied and differences in their morphologies, ecologies, and life histories are well established [25-28]. The morphological differences between these two ecotypes consist of numerous traits that influence the foraging efficiency of fish feeding in different habitats and on different types of prey [25, 26]. Benthic fish typically have deeper heads and bodies, fewer more widely spaced gill rakers, more robust cranial morphology, and smaller eyes. Limnetic ecotypes have traits that are well suited for feeding on planktonic prey including more fusiform body shape, a longer narrower snout, numerous fine gill rakers, and relatively large eyes. These morphological differences are correlated with several ecological traits including diet, trophic position, and habitat use [14, 25, 29]. Additionally, in sympatric populations, benthic and limnetic stickleback differ in their reproductive allocations [28], behavior, and nesting sites [26, 27]. The major differences between benthic and limnetic stickleback are maintained under common conditions and hybrids are intermediate, indicating that these traits are heritable [26, 29]. Further, there

is differential fitness of the ecotypes in their respective habitats [26, 29], indicating that the distinction between ecotypes is ecologically and evolutionarily relevant.

Benka Lake is the only lake in Alaska known to have both benthic and limnetic ecotypes of stickleback [27-29]. The Benka ecotypes have distinct morphologies, diets, and life histories similar to those seen in other sympatric populations of benthic and limnetic stickleback, though the Benka ecotypes are less differentiated [27-29]. In order to understand the role of habitat specific foraging in determining Hg concentrations of stickleback from Benka Lake, I measured total Hg (THg) and stable isotope ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) in reproductive (age 2) females and males of both ecotypes. Specifically, isotope data were used to estimate TP and percent benthic carbon utilized (α). Levels of THg, TP and α were used to address three questions: 1) Do THg concentrations vary between ecotypes? 2) Do THg concentrations vary between sexes? 3) How important is α relative to TP? This study provides a means of examining the relative importance of α and TP in the accumulation of THg in a single population, and establishes the foundation for future studies examining the relative importance of these factors in comparison to inter-population variation.

2.4 Materials and Methods

2.4.1 *Study site*

Benka Lake (62.1875° N, 150.0040° W) is a relatively small (< 0.5 km^2) landlocked lake located approximately 125 km north of Anchorage in the Cook Inlet Basin of Alaska (Fig. 2.1). Benka Lake has several shallow bays, which provide benthic

habitats, and a central basin, which provides limnetic habitats. The central basin is deep (maximum depth = 23 m) compared to similar sized lakes in the region, and there are many areas where the shoreline descends steeply into deeper waters [27, 30].

2.4.2 Sample collection

Stickleback were collected in July, 2010 from eight areas of Benka Lake that had previously been identified as breeding areas for either benthic or limnetic ecotypes (Fig. 2.1) [27]. Stickleback were collected using unbaited 0.64 cm wire mesh minnow traps set near shore. While all traps were on bottom and approximately the same distance from shore, in benthic areas traps were set at depths < 0.5 m whereas traps in limnetic sites were often 1-2 m deep. Fish were designated as "benthic" or "limnetic" depending upon collection site [27-29] and differences in cranial morphology (J. Willacker, University of Alaska Anchorage, Anchorage, AK, USA). Fish were euthanized with an overdose of buffered MS-222 anesthetic (Argent Laboratories), rinsed in lake water, and stored on crushed dry ice while in the field (2-8 hours), then at -80°C in the laboratory. In order to account for differences in the isotopic baselines of benthic and limnetic food webs, eight gastropods (*Helisoma anceps*) and eight mussels (*Anodonta beringiana*) were collected from the same areas as stickleback and preserved in the same manner.

2.4.3 Sample preparation

Analyses utilized 40 adult fish of each sex from each ecotype except for limnetic males, which had a sample size of 39 (total n = 159). To minimize errors in sex assignment, only reproductively mature fish (based on gonad dissections and secondary sexual characteristics) were used. Use of reproductive fish also reduced variation in age

since the vast majority of reproductive stickleback are age 2 [26, 28]. For each fish, standard length (SL; anterior tip of premaxilla to posterior border of hyperal plate) was measured to the nearest millimeter, and a unique specimen identification number was assigned. The head of each specimen was then removed with a cut directly behind the operculum for a separate study of cranial morphological divergence in Benka Lake stickleback. The exclusion of heads did not alter isotope ($t = -0.53$, degrees of freedom [*df*] = 58, $p = 0.591$) or Hg ($t = 0.68$, *df* = 58, $p = 0.496$) values compared to those of whole (head-on) homogenates of stickleback (n = 30). Thus, all further preparation procedures and analyses are for the headless carcasses. In addition, all macro-parasites (*Schistocephalus solidus* and Anisakidae), eggs, and stomach contents were removed to prevent biases due to the presence of unassimilated materials and the high lipid content of eggs (mean lipid content of eggs = 10.5% dry weight [dw], n = 36).

Headless stickleback carcasses were freeze-dried for 72 hr. then ground into a fine powder using a Beadbeater tissue mill (Bio Spec Products Inc.) using 3.2 mm stainless steel beads. Gastropod and mussel samples were removed from their shells and freeze-dried for 144 hr. (due to their larger volumes) before homogenization in the tissue mill.

2.4.4 Stable isotope analysis

For each sample, approximately 0.5 mg of dried homogenate was sealed in a tin capsule and analyzed for stable carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) isotope ratios at the University of Alaska Anchorage – Environmental and Natural Resources Institute Stable Isotope Laboratory using a Thermo-Finnigan Delta Plus XP Isotope Ratio Mass Spectrometer (IRMS) coupled to a Costech 4010 Elemental Combustion System. Isotope

ratios are presented in standard δ notation as parts per thousand (‰) differences between the isotope ratio of the sample and that of a standard (Vienna Pee Dee Belemnite for carbon and air for nitrogen). Samples depleted in the heavier isotope (^{13}C or ^{15}N) in comparison to the standard have lower delta values. All instruments are regularly calibrated using a suite of International Atomic Energy Agency standards to ensure instrument precision, accuracy, and tuning parameters are to specification. At least six replicates each of a stable isotope reference material (SRM 1547: peach leaves, available from the National Institute of Standards and Technology; $\delta^{13}C = -25.89 \pm 0.3‰$, $\delta^{15}N = 1.90 \pm 0.3‰$) and laboratory working standard (Cheney Lake stickleback; $\delta^{13}C = -29.50 \pm 0.2‰$, $\delta^{15}N = 10.13 \pm 0.1‰$) were analyzed with every batch of 40 samples. External instrument reproducibility for both carbon and nitrogen isotope analysis was ± 0.2‰. Isotope values were lipid normalized for a subset of samples (n = 40), but because lipid content was uniformly low in carcasses (lipid of males and females with eggs removed = 2.1 ± 0.7%; C:N = 3.34 ± 0.3), normalization had minimal impact and non-normalized values are presented.

2.4.5 Mercury analysis

THg concentrations in stickleback were measured in approximately 26 ± 4 mg of dried headless carcass homogenate at the Wildlife Toxicology Laboratory, University of Alaska Fairbanks using a DMA-80 Direct Mercury Analyzer (Milestone Inc.). Quality assurance/quality control followed standard laboratory procedures and included duplicate method blanks, spiked blanks, and standard reference materials run with each batch of 20 samples. The standard reference material consisted of fish protein homogenate (DORM-

3; THg = 382 ± 60 ng/g) obtained from the National Research Council of Canada. Recovery rates for spiked blanks ranged from 88 to 97%. All samples were initially analyzed in duplicate and when the relative standard deviation between two replicates was greater than 10% additional replicates were run. Mercury data are presented on a dry weight basis, but can be converted to wet weight (ww) estimates using the formula

$$[THg]_w = [THg]_d \times (1-(0.01 \times PM))$$

where $[THg]_w$ is the estimated wet weight THg concentration, $[THg]_d$ is the measured dry-weight THg concentration, and PM is the mean percent moisture of stickleback used in this study (75.7% ± 0.02%, n = 68).

2.4.6 Statistical analysis

In order to assess the importance of trophic ecology as a determinant of THg concentration, I calculated the proportion of carbon from benthic sources (α) in the diet of individual stickleback using the equation in Post [12]

$$\alpha = (\delta^{13}C_{stickleback} - \delta^{13}C_{lim})/(\delta^{13}C_{ben} - \delta^{13}C_{lim})$$

where $\delta^{13}C_{lim}$ is the $\delta^{13}C$ value of the limnetic baseline (mussels) and $\delta^{13}C_{ben}$ is the $\delta^{13}C$ value of the benthic baseline (gastropods). I also calculated the TP of stickleback following Post [12]

$$TP_{stickleback} = \lambda_{base} + (\delta^{15}N_{stickleback} - [\delta^{15}N_{ben} \times \alpha + \delta^{15}N_{lim} \times (1 - \alpha)])/\Delta_N$$

where λ_{base} is the TP of the consumers that are serving as the isotopic baselines ($\lambda_{base} = 2$), $\delta^{15}N_{ben}$ is the $\delta^{15}N$ value of the benthic baseline, $\delta^{15}N_{lim}$ is the $\delta^{15}N$ value of the limnetic baseline, and Δ_N is the trophic enrichment between consumers. In this study a Δ_N of 2.63 was used based on observed trophic fractionation in controlled feeding experiments with

threespine stickleback from two nearby populations (J. Willacker, University of Alaska Anchorage, Anchorage, AK, USA). When a generalized Δ_N of 3.4 is utilized the results are qualitatively the same (J. Willacker, University of Alaska Anchorage, Anchorage, AK, USA).

Prior to analysis, data were examined to ensure they met the underlying assumptions of parametric statistics. Mercury data were natural-log transformed to meet the assumption of homogeneity of variances and geometric means are presented unless otherwise noted. Differences in SL, $\delta^{13}C$, α, $\delta^{15}N$, TP, and THg between ecotypes were initially assessed using two sample *t*-tests. After establishing that the ecotypes differ, I followed a more refined approach of using α as a measure of resource use because α is a continuous, quantitative variable that accounts for ecological variation within ecotypes as well as differences between ecotypes [14]. For analyses that could be conducted in common using either ecotype as a fixed factor or α as an independent variable, the results were qualitatively the same; therefore, I present model results that employed α as an independent variable rather than ecotype as a fixed factor. Linear regression coupled with quantitative model selection techniques were used to examine the effects of sex, SL, α, and TP on THg concentrations. Sample size corrected Akaike's Information Criterion (AIC_C) was used to select the most parsimonious model for explaining THg concentrations from a set of *a priori* candidate models [31]. A systematic AIC_C approach was used to first compare 34 candidate models including all possible main-effects combinations of sex, α, TP, and SL, as well as interactions between sex and each covariate (Table 2.S1).

Differences between the AIC_C of the best model and the other candidate models (ΔAIC_C) and the Akaike weights (W) of candidate models were compared to assess each model's probability of being the best fitting model [31]. Interpretation of main-effects was complicated by interactions between sex and the covariates in the top model. Therefore, models were split by sex and a set of eight candidate models were compared for each sex. These second order models were assessed in the same way as the first order models. In addition, partial regressions were used to assess the relative importance of each variable in the top models. Analysis of covariance was not employed because co-linearity between α and TP violates the assumption of independent covariates. All analyses were conducted in R version 2.9.2 [32].

2.5 Results

Benthic and limnetic primary consumers in Benka Lake were distinct in their $\delta^{13}C$ values with limnetic mussels isotopically depleted compared to benthic gastropods (-35.0 ‰ and -20.1‰, respectively; t = 24.83, $df = 14$, $p < 0.001$). The two ecotypes of stickleback were significantly differentiated in all variables measured (Table 2.1). Stickleback ranged in SL from 44 to 67 mm, with benthic fish being larger on average than limnetic fish (Table 2.1; t = 3.83, $df = 157$, $p < 0.001$). The isotopic values of stickleback reflected those of the primary consumers of the habitat in which they were captured, with limnetic individuals having depleted $\delta^{13}C$ values compared to fish from the benthic habitat (Table 2.1; t = 2.94, $df = 157$, $p = 0.004$). Calculated α values for individuals ranged from 0.04 to 0.83 and again fish captured from the limnetic habitat

had a lower mean α than fish from the benthic habitat (Table 2.1; t = 2.94, *df* = 157, *p* = 0.004). The TP of individuals ranged from 3.56 to 4.86 and fish of the limnetic ecotype had on average higher $\delta^{15}N$ (t = -4.27, *df* = 157, *p* < 0.001) and thus TP (t = -4.50, *df* = 157, *p* < 0.001) than fish of the benthic ecotype (Table 2.1).

Mean Hg concentrations were 16.1% higher in limnetic stickleback than in benthic stickleback (Table 1; t = -3.15, *df* = 157, *p* = 0.002). Overall, male stickleback had 29.4% higher mean THg concentrations than female stickleback (mean THg = 198 and 153 ng/g dw for males and females respectively; t = -6.38, *df* = 157, *p* < 0.001). The difference between sexes was maintained in both benthic (t = -4.90, *df* = 78, *p* < 0.001) and limnetic (t = -4.50, *df* = 77, *p* < 0.001) ecotypes (Fig. 2.2). THg concentrations were not associated with fish size (SL; r = 0.12, n = 159, *p* = 0.158).

The most parsimonious model explaining THg concentrations in stickleback included sex, α, and TP as main effects (ΔAIC_C for all other models > 2; Table 2.S1). The best fitting model also included interactions between sex and both covariates, suggesting that both covariates differed in their relationship with THg for female and male fish. Subsequent assessment of ΔAIC_C and Akaike's weights in sex specific models indicated a high probability that the top model was the most parsimonious (*W* = 0.649 and *W* = 0.708 for females and males, respectively); both the female and male models included both α and TP as main effects (Table 2.2). However, the treatment of α in the models differed by sex. In particular, males demonstrated a slight positive relationship between THg and α, while females demonstrated a negative relationship. In both sexes TP was positively correlated with THg, though the coefficients differed.

Due to a high degree of co-variation between α and TP, partial regressions were used to assess the relative importance of each variable to the models (Fig. 2.3). Partial regressions indicated that in females TP was only slightly more important than α in determining THg concentration (partial regression coefficient, $\eta^2 = 0.101$ and 0.057 for TP and α, respectively). In contrast to females, the partial regressions of the male model demonstrate that TP is clearly more important than α ($\eta^2 = 0.217$ and 0.053 for TP and α, respectively). Variable weights supported these conclusions and showed that in females α and TP were approximately equal in importance ($V = 0.858$ and 0.903, respectively) while in males TP ($V = 1.000$) was a more important factor than α ($V = 0.706$).

2.6 Discussion

Stickleback occupy middle TPs in most food webs and are important prey for many piscivorous fishes and birds. Thus, additional Hg biomagnification will occur at TPs above that of stickleback. Canada [33] and California [34] have both set a Hg advisory level of 33 ng/g ww for forage fish. This value incorporates the potential for additional biomagnification in piscivorous wildlife and is thus a conservative guide for assessing Hg levels in forage fish. In Benka Lake, the overall mean THg concentration in stickleback was over this advisory level; however, segregated by sex and ecotype, benthic females had THg concentrations that were not significantly different than the advisory level (single sample t-test: t = -0.51, $df = 39$, p = 0.615) while THg concentrations in males of both ecotypes and limnetic females were significantly higher than the advisory level ($p \leq 0.002$). Thus, while the differences in Hg concentrations reported in the present study are

small, they may be large enough to impact the vulnerability of some sensitive wildlife species, especially long-lived, high-trophic level consumers. These results underscore the importance of both sex and food web utilization in determining Hg accumulation in higher trophic levels.

The limnetic ecotype of stickleback in Benka Lake had a significantly higher (16.1%) concentration of THg than the benthic ecotype. Differentiation in the Hg concentrations of trophically divergent species and populations has been examined in a variety of systems [1, 2, 16, 17-24], though to date, few studies have examined the role of habitat specific foraging in a single species from a single system (but see Chumchal et al. [17]). As in the present study, Hg concentrations in limnetic feeding fish are typically higher than corresponding concentrations in fish utilizing benthic habitats.

Two explanations for this trend have been proposed. First, limnetic food webs may have more bioavailable Hg at low TPs or Hg may be more efficiently accumulated from limnetic habitats. Phytoplankton are known to bioconcentrate Hg at a much higher rate than benthic algae [35] and often can have Hg concentrations thousands of times greater than the surrounding water [22, 23]. In addition, zooplankton and littoral macroinvertebrates differ in their feeding specificities. While zooplankton primarily consume bacteria and phytoplankton of autochthonous origin, inputs of terrestrial and lake detritus are a major source of energy for littoral macroinvertebrates. Because detrital materials do not actively incorporate new Hg from the water, use of these resources could reduce the incorporation of environmental Hg into the littoral food web [24]. As a result of these factors, differences in Hg concentration have also been observed between

limnetic zooplankton and littoral associated macroinvertebrates [24] and between limnetic and littoral associated zooplankton [36]. Furthermore, Paterson et al. [37] found that MeHg+ concentrations in zooplankton increased more rapidly and to a greater extent than in littoral macroinvertebrates following experimental increases in the production of MeHg+. These data support the hypothesis that higher THg concentrations in limnetic feeding fish are driven by increased uptake of Hg into limnetic food webs compared to benthic food webs.

Another explanation for higher Hg concentrations in limnetic food webs relates to differing biological conditions in the two food webs. Specifically, differences in size, age, productivity, growth rates, and TP are known to alter the concentration of Hg in fish. Many studies have demonstrated that Hg biomagnifies through food webs such that individuals with higher TPs often have substantially higher Hg concentrations than individuals at lower TPs. The biomagnification of contaminants in a food web is related to the number of trophic transfers in the web (i.e., food chain length) [15] as well as the assimilation efficiency of the consumers. The rate of Hg assimilation in consumers does not vary widely between lake food webs [2, 10 16]; however, limnetic food webs typically have more trophic transfers and a greater degree of omnivory (i.e., consumers feeding on multiple trophic levels) than littoral food webs [13, 15]. Thus, higher Hg concentrations in limnetic foraging fish could result from higher TPs in limnetic habitats compared to benthic habitats.

While this mechanism may be an important determinant of biomagnification for other contaminants [11], few studies have found support for TP driving benthic-limnetic

differentiation in Hg concentrations. In most studies, differences in benthic and limnetic Hg concentrations were associated with differing Hg availability to low trophic levels rather than differences in the TP of consumers or biomagnification of Hg through a food web [1, 16-19, 22, 24]. For example, Ethier et al. [1] found that limnetic feeding yellow perch (*Perca flavescens*) had higher Hg concentrations than benthic feeding pumpkinseed sunfish (*Lepomis gibbosus*) despite the similarity in their TPs.

In the present study, benthic and limnetic stickleback differed in their TPs with the limnetic ecotype having a higher mean TP than the benthic ecotype. Multiple regression and AIC indicate that both α and TP are important determinants of THg concentrations in Benka Lake stickleback; however, the two variables were strongly co-linear. Thus, I used variable weights and partial regressions to examine the relative importance of the two variables. While results differed between the sexes, TP was a more important determinant of THg concentration than α in both female and male stickleback. The higher relative importance of TP compared to α is consistent with the results of the only other study of habitat-specific Hg accumulation in the sub-Arctic [21], but differs from studies conducted in more temperate to tropical regions [1, 16-19, 22, 24].

Power et al. [21] suggested that the increased importance of TP (approximated as $\delta^{15}N$) observed in Stewart Lake, Nunavik, Canada compared to more temperate lakes might be due to reduced growth rates. Growth rate is known to influence Hg concentration by regulating the dilution of Hg in tissue [4, 35]. Differences in the growth rates of benthic and limnetic foraging fish could provide a mechanism to explain increased biomagnification rates in the limnetic versus benthic food webs of Benka Lake

where shallow benthic habitats warm faster in the spring and are typically 4-6°C warmer than deep limnetic habitats (Fig. 2.S1). I found that adult benthic stickleback in Benka Lake are significantly larger than limnetic stickleback of the same age, consistent with temperature differences in the two habitats, but also possibly due to foraging differences. Cresko [29] found that stickleback confined to benthic habitats of Benka Lake grew faster than those confined in limnetic habitats. Baker et al. [28] also suggest that temperature and resulting differences in growth rates are responsible for divergent reproductive strategies in benthic and limnetic females from Benka Lake. In sum, the lower observed Hg concentration in benthic stickleback from Benka Lake could be due to growth dilution resulting from either temperature or foraging effects.

While α was a significant determinant of THg concentrations in Benka Lake stickleback, I did not find as strong of a correlation as has been observed in other systems [1, 16-22, 24]. This is expected considering that I parsed variation between TP and α rather than relying on simple regressions between THg and each covariate, which over-estimates the variance explained [31]. Additionally, I examined a single species within a single lake while most studies have examined multiple species in a single lake or a single species across multiple lakes. Although the two ecotypes in Benka Lake are morphologically and ecologically divergent, they represent modal phenotypes across a relatively narrow continuum of differentiation and thus overlap in their morphological and ecological distributions [27-29]. The mean level of benthic carbon differed significantly between the ecotypes, but the difference was modest (36% vs. 29% for benthic and limnetic fish, respectively; Table 1). Thus, both ecotypes in Benka Lake

heavily utilize limnetic resources. This small but significant difference in diet mirrors differences in the morphologies of the two ecotypes, and supports the conclusion that the Benka Lake ecotypes are less differentiated than sympatric benthic-limnetic species pairs of stickleback in British Columbia or some allopatric populations throughout the Cook Inlet Basin [27-29]. Considering the modest extent of dietary differences in Benka Lake stickleback, it is not surprising that the relationship between THg concentration and resource utilization is reduced compared to this relationship for allopatric populations or different species.

In both ecotypes, females had lower THg concentrations than males. Sex differences were greater than differences between ecotypes. The relationship between THg concentration, TP and α differed in the two sexes. Partial regressions and variable weights indicate that in males TP is a more important determinant of THg concentration than α. In females, although TP is more important than α, the difference is modest and both variables displayed low partial regression coefficients. The seemingly low explained variance of the two variables in females is likely a reflection of the similar but opposite partial regression coefficients, which result in the underestimation of variance assigned to each variable [38].

Similar trends have been observed in several other species, and in some species sex is the primary factor governing Hg bioaccumulation [6, 7]. However, little agreement exists on the mechanisms underlying these differences. In many species of fish, including threespine stickleback, females and males are often ecologically differentiated [39]. In some populations of stickleback, the sexes are known to differ in their habitat use,

feeding behavior, and trophic morphology [26]. In the present study, the sexes were ecologically distinct with females of both ecotypes utilizing more benthic carbon than males. Furthermore, the pattern of isotopic divergence between the sexes mirrored differences in THg concentrations, suggesting that trophic differences may account for the observed differences in THg between the sexes. However, the different relationships between THg concentration, TP, and α in males and females indicate that sexual differences in THg are not a simple matter of the sexes occupying different positions along the benthic-limnetic axis. Thus, additional factors likely contribute to sexual differences in THg concentration.

It has been suggested that lower Hg concentrations in female fish are due to the loss of Hg in eggs [6, 9, 40]. However, Hg concentration in females of several fish species increases following egg deposition [8]. Furthermore, Niimi [8] and others [6, 7, 40] showed that the Hg concentration of eggs is only 0.3% to 2.3% of an individual's body concentration, and concluded that spawning is not sufficient to explain differences in Hg concentration between the sexes. However, in Benka Lake stickleback, egg THg ranged from 34 to 45 ng/g dw (n = 60) which is 21.7 to 24.7% of the mean concentration in the body in both ecotypes (J. Willacker, University of Alaska Anchorage, Anchorage, AK, USA). Additionally, stickleback produce multiple egg clutches during the breeding season [26], which may result in high net loss despite relatively low loss in each clutch. Therefore, my Benka Lake data suggest that egg sequestration could be a more important driver of female-male Hg differences than in other species. Additionally, these studies have only examined changes in tissue concentrations rather than burdens. Thus, changes

in the total amounts of Hg in female tissues across the breeding season have not been examined. Furthermore, these studies generally treated fish as a single compartment and did not separately examine Hg burdens in the somatic and reproductive tissues. Such differentiation could provide important insights on the distribution of Hg in fish tissues and the mechanisms generating sexual differences.

Higher THg concentrations in males could also be the result of slower growth rates and thus reduced growth dilution of Hg. Differential growth rates have been implicated as a potential cause of sex based differences in the Hg concentrations of several fish taxa including northern pike (*Esox lucius*) [3], sharks [5], sunfish [9], and lake trout (*Salvelinus namaycush*) [7]. In stickleback, parental care is provided by males, and this care is energetically expensive [26]. Additionally, male stickleback are restricted to foraging near their nest during the breeding season, while females forage over large areas and prey upon energetically productive eggs [26]. Therefore, it is plausible that growth rates differ between the sexes, contributing to differences in THg concentration.

My results demonstrate that both sex and habitat-specific foraging can be important determinants of THg concentrations in a single threespine stickleback population. Further, I found that the relationships between THg concentration, TP, and α differed between the sexes such that TP and α were of approximately equal importance in female fish but TP was more important than α in male fish. My data suggest that sex may play a critical role in determining Hg concentrations and modifying the roles of ecological factors, and therefore that the sexes should be examined separately in fish Hg studies.

Additionally, the mechanisms underlying sexual differentiation of Hg levels need to be further examined.

The threespine stickleback species complex provides a valuable model for studying details of Hg ecology. In particular, the differentiation between benthic and limnetic feeding stickleback, both within and between lakes, provides an opportunity to examine the relative importance of habitat-specific foraging in relation to lake and watershed scale variables. Additionally, stickleback are easily reared under a variety of laboratory conditions and are thus amenable to captive studies examining the roles of individual environmental factors, their interactions, and the mechanisms by which these factors influence Hg accumulation in fish. Collectively, such field and laboratory studies can improve our understanding of Hg dynamics in aquatic systems and provide valuable insights into the factors governing Hg accumulation in higher trophic levels.

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2.9 Tables

Table 2.1: Mean variable values for benthic and limnetic ecotypes of stickleback from Benka Lake, Alaska. Geometric and arithmetic means are presented for total mercury (THg), arithmetic only for others. Standard deviations are in parentheses. p values indicate results of t-tests for significance between ecotypes.

Parameter	Benthic (n = 80)	Limnetic (n = 79)	*p*-value
Standard length (mm)	57.64 (3.9)	54.75 (5.1)	< 0.001
$\delta^{13}C$ (‰)	-29.56 (2.7)	-30.73 (2.2)	0.004
$\alpha^{\dagger}$	0.36 (0.2)	0.29 (0.2)	0.004
$\delta^{15}N$ (‰)	8.43 (1.2)	9.11 (0.8)	< 0.001
TP‡	4.22 (0.3)	4.43 (0.2)	< 0.001
THg (ng/g dw)			
Geometric	156 (30)	181 (30)	0.002
Arithmetic	162 (50)	188 (50)	0.002

† The proportion of dietary carbon from benthic sources

‡ Trophic position

Table 2.2: Structure and evaluation criteria for candidate models describing natural log transformed total mercury concentrations (lnTHg) in female and male stickleback from Benka Lake, Alaska.

Model	Model Structure	n	R^2	AIC_C[a]	ΔAIC_C[b]	W[c]
Females						
1	lnTHg ~ α + TP + SL	80	0.30	11.30	3.86	0.09
2*	lnTHg ~ α + TP	80	0.31	7.43	0.00	0.65
3	lnTHg ~ α + SL	80	0.23	17.71	10.27	0.00
4	lnTHg ~ TP + SL	80	0.29	12.14	4.71	0.06
5	lnTHg ~ α	80	0.24	13.97	6.54	0.02
6	lnTHg ~ TP	80	0.28	10.15	2.72	0.17
7	lnTHg ~ SL	80	0.00	37.09	29.66	0.00
8	Null	80	0.00	35.08	27.65	0.00
Males						
1	lnTHg ~ α + TP + SL	79	0.22	-10.88	3.41	0.13
2*	lnTHg ~ α + TP	79	0.20	-14.29	0.00	0.71
3	lnTHg ~ α + SL	79	0.02	3.53	17.82	0.00
4	lnTHg ~ TP + SL	79	0.15	-6.11	8.18	0.01
5	lnTHg ~ α	79	0.00	3.01	17.30	0.00
6	lnTHg ~ TP	79	0.15	-11.19	3.09	0.15
7	lnTHg ~ SL	79	0.03	1.56	15.85	0.00
8	Null	79	0.00	1.09	15.38	0.00

[a] Sample size corrected Akaike's Information Criterion
[b] The difference between the current model AIC_C and the AIC_C of the most parsimonious model
[c] Akaike's weight; the likelihood of the current model relative to others in the candidate set
* the most parsimonious candidate model for each sex
α = proportion benthic carbon
TP = trophic position
SL = standard length

2.10 Figures

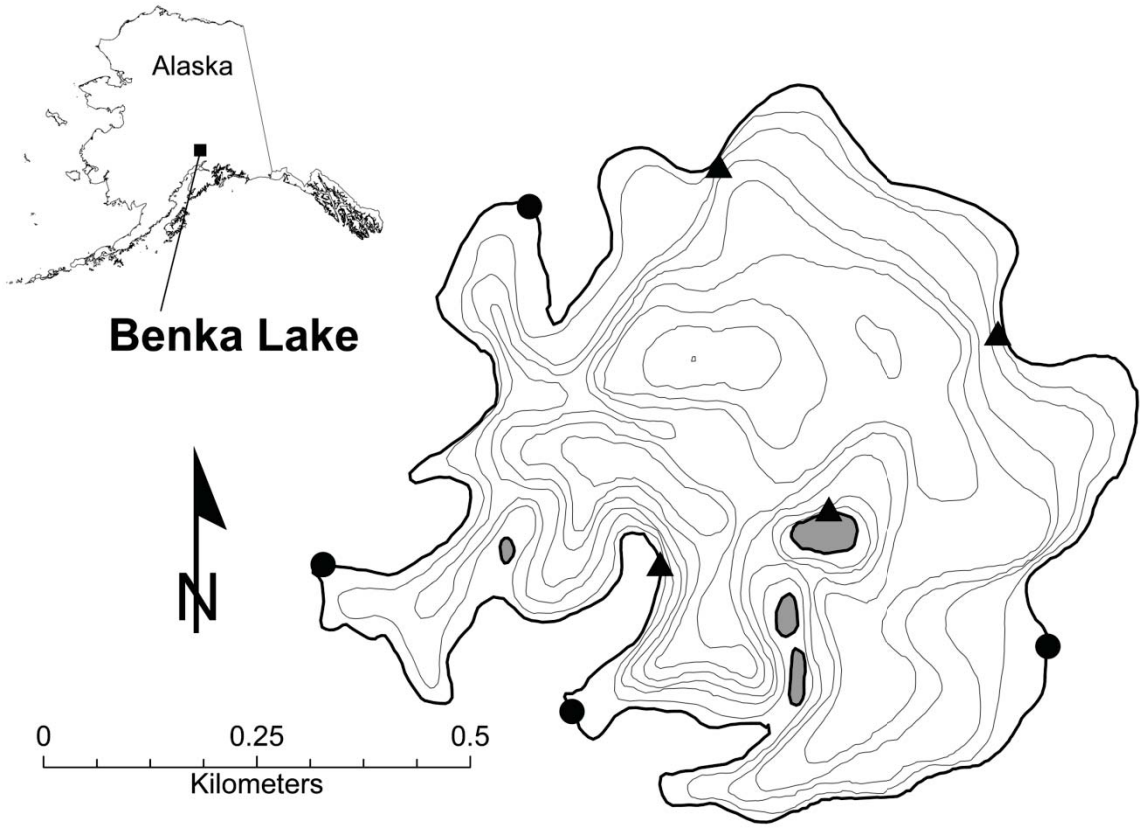

Figure 2.1: Map of Benka Lake, Alaska indicating benthic (circles) and limnetic (triangles) sampling sites. Depth contours are in 3.05 m increments. Grayed areas are islands.

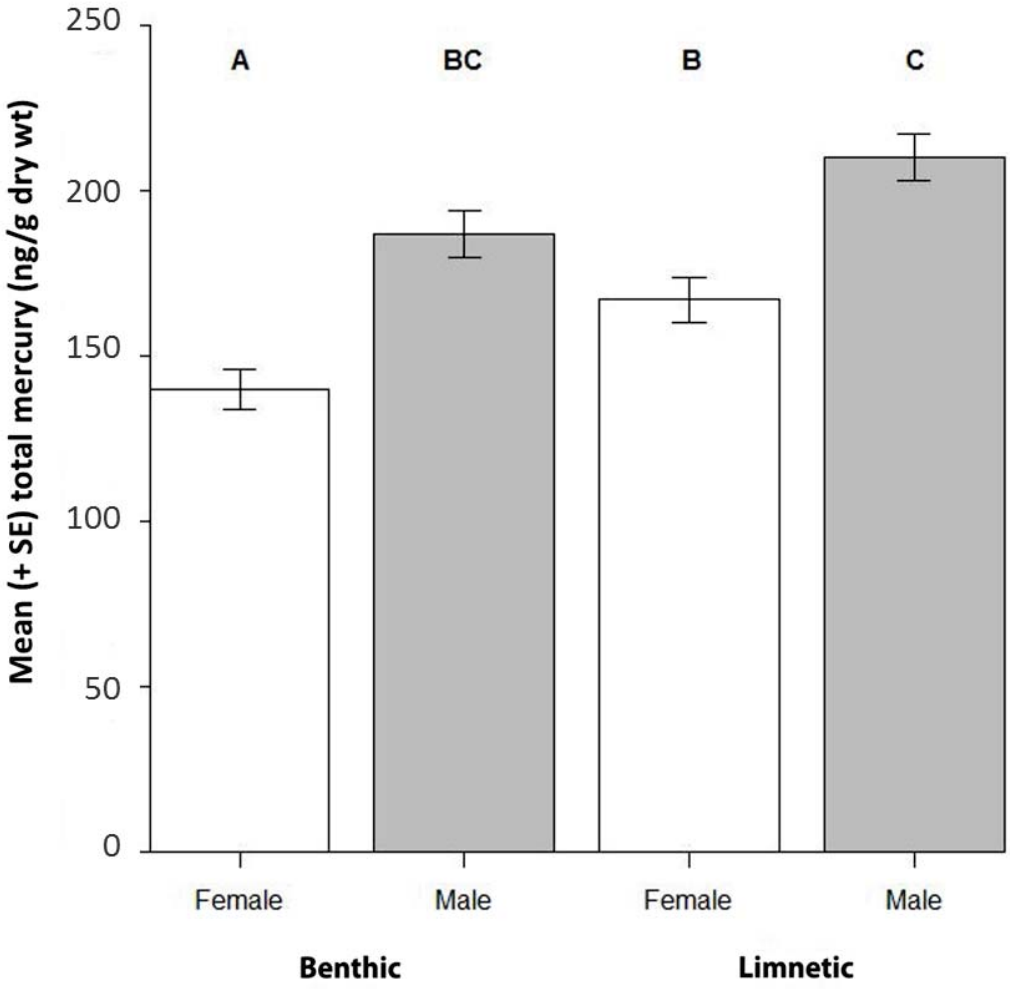

Figure 2.2: Mean total mercury concentrations in stickleback from Benka Lake, Alaska by ecotype (benthic – limnetic) and sex (female – male). Bold letters designate group means that are statistically different ($p < 0.05$).

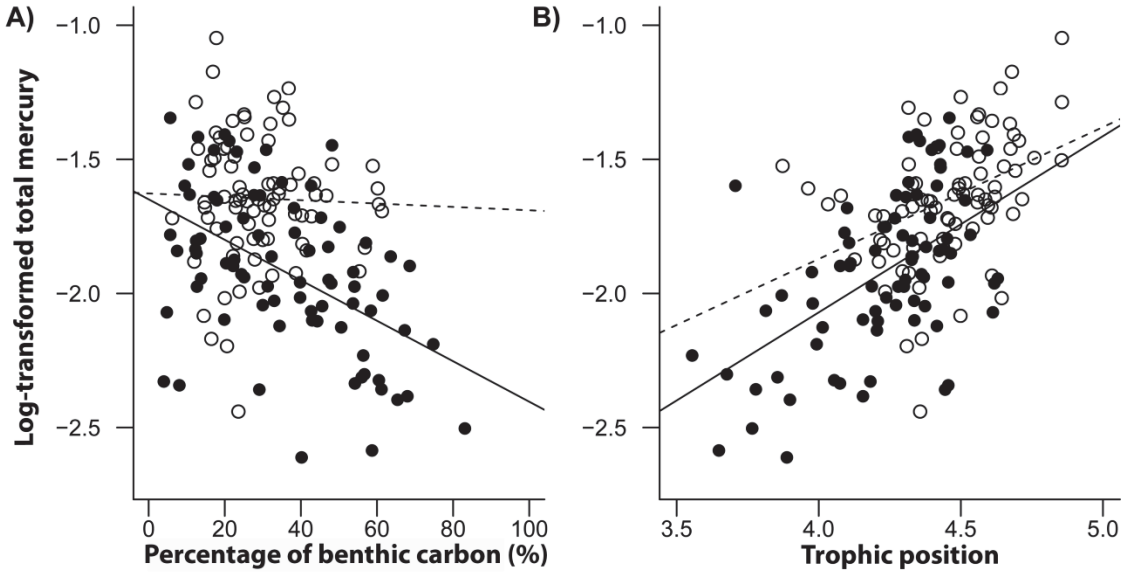

Figure 2.3: Relationships between total mercury and A) percent benthic carbon (α) or B) trophic position in stickleback from Benka Lake, Alaska. Solid points and lines indicate females, open points and dashed lines indicate males.

2.11 Supplemental Information

Table 2.S1: Structure and evaluation criteria for candidate models describing total mercury concentrations in stickleback from Benka Lake, Alaska. Models include additive (+) and interaction (*) terms. Variables are coded as: Sex = Sex, α = proportion benthic carbon, TP = trophic position, SL = standard length. Models with a ΔAIC_C value < 2 are indicated in bold.

Model Structure	N	R^2	AIC_C[a]	ΔAIC_C[b]	W^C[c]
Sex	159	0.20	37.74	45.90	0.00
α	159	0.13	49.02	57.19	0.00
TP	159	0.33	5.85	14.02	0.00
SL	159	0.01	64.40	72.56	0.00
Sex+α	159	0.28	20.03	28.19	0.00
Sex+TP	159	0.37	-2.69	5.47	0.03
Sex+SL	159	0.18	39.31	47.47	0.00
α+TP	159	0.33	7.73	15.89	0.00
α+SL	159	0.12	47.42	55.58	0.00
TP+SL	159	0.31	10.43	18.59	0.00
Sex+α+TP	159	0.37	-1.39	6.77	0.02
Sex+α+SL	159	0.25	25.62	33.78	0.00
Sex+TP +SL	159	0.35	5.03	13.19	0.00
α+TP+SL	159	0.31	12.30	20.46	0.00
Sex+α+TP+SL	159	0.35	7.00	15.16	0.00
Null	159	0.00	72.43	80.59	0.00
Sex+α+TP+SL+(Sex*α)	159	0.36	1.79	9.95	0.00
Sex+α+TP+SL+(Sex*TP)	159	0.35	7.76	15.92	0.00
Sex+α+TP+SL+(Sex*SL)	159	0.36	6.83	14.99	0.00
Sex+α+TP+SL+(Sex*α)+(Sex*TP)	159	0.37	3.26	11.43	0.00
Sex+α+TP+SL+(Sex*α)+(Sex*SL)	159	0.39	-0.95	7.21	0.01
Sex+α+TP+SL+(Sex*α)+(Sex*TP)+(Sex*SL)	159	0.40	0.28	8.44	0.01
Sex+α+TP (Sex*α)	**159**	**0.39**	**-8.16**	**0.00**	**0.53**
Sex+α+TP (Sex*TP)	159	0.38	-0.10	8.06	0.01
Sex+α+TP+(Sex*α)+(Sex*TP)	**159**	**0.41**	**-7.36**	**0.80**	**0.36**
Sex+α+SL+(Sex*α)	159	0.27	21.44	29.60	0.00
Sex+α+SL+(Sex*SL)	159	0.26	26.07	34.24	0.00
Sex+α+SL+(Sex*α)+(Sex*SL)	159	0.29	19.92	28.08	0.00
Sex+TP+SL+(Sex*TP)	159	0.35	5.76	13.92	0.00
Sex+TP+SL+(Sex*SL)	159	0.36	4.90	13.07	0.00
Sex+TP+SL+(Sex*TP)+(Sex*SL)	159	0.36	5.26	13.42	0.00
Sex+α+(Sex*α)	159	0.29	15.36	23.52	0.00
Sex+TP+(Sex*TP)	159	0.37	-1.61	6.56	0.02
Sex+SL+(Sex*SL)	159	0.18	40.69	48.85	0.00

[a] Sample size corrected Akaike's Information Criterion
[b] The difference between the current model AIC_C and the AIC_C of the most parsimonious model
[c] Akaike's weight; the likelihood of the current model relative to others in the candidate set

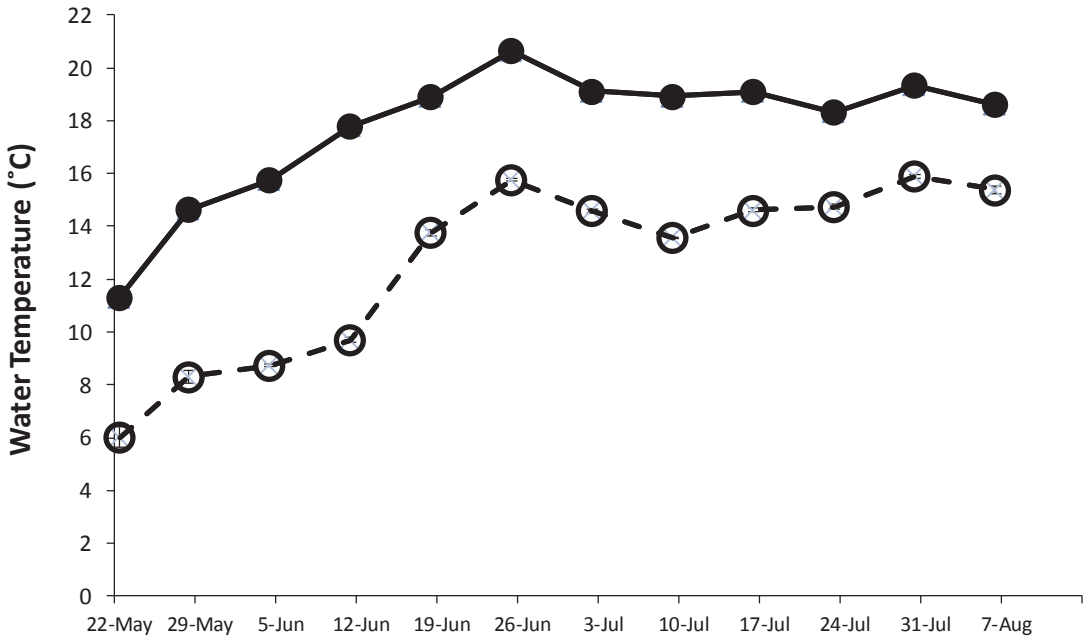

Figure 2.S1: Water temperature at stickleback spawning areas in Benka Lake, Alaska during the 2012 breeding season. Benthic sites are represented by solid points and line, limnetic sites by open points and dashed line. Each point is the average from four sites. Standard errors for the measurements were very low and thus error bars are not visible in the figure.

Chapter 3: Ecological correlates of mercury accumulation in threespine stickleback fish[1]

3.1 Abstract

The bioaccumulation of mercury in fishes is a complex process influenced by numerous chemical, physical, ecological, and physiological factors at multiple hierarchical levels. I utilized threespine stickleback fish (Gasterosteus aculeatus) to examine the relative importance of various biological factors in governing mercury accumulation within and among six populations ranging from extreme benthic to extreme limnetic ecologies. Across these populations, I found that sex and trophic position were significantly more important than habitat-specific foraging; however, there was substantial variation in the relative importance of these parameters in individual lakes. I also found a positive correlation between total mercury concentrations and reliance on benthic prey when examined across populations. When variation in mercury concentrations of primary consumers was accounted for there was no relationship between total mercury concentrations and reliance on benthic prey. These findings suggest that patterns of stickleback mercury concentrations at a landscape scale are driven by factors regulating mercury bioavailability, while habitat-specific foraging and trophic position are more likely to play roles in determining within-population patterns of Hg concentrations.

[1] Willacker, J. J., C. A. Eagles-Smith, F. A. von Hippel, and T. M. O'Hara. Ecological correlates of mercury accumulation in threespine stickleback fish. Prepared for submission to *Environmental Toxicology and Chemistry*.

3.2 Keywords

benthic, ecotypes, *Gasterosteus aculeatus*, habitat-specific foraging, limnetic, trophic position

3.3 Introduction

Mercury (Hg) is a pervasive contaminant of natural systems found in nearly all environmental samples [1]. In its methylated forms (methylmercury; MeHg+), Hg poses developmental and reproductive adverse health risks in exposed wildlife and people. Mercury is a particular concern in aquatic environments where it is often found in elevated concentrations, is known to bioaccumulate in individuals and biomagnify in food webs, and in the United States is responsible for most fish consumption advisories [2]. In many taxa, including fish, birds, and mammals, the consumption of aquatic biota is the primary route of exposure to MeHg+ even in cases where aquatic prey comprise a relatively small proportion of the diet [3-6].

Environmental Hg contamination occurs as the result of both natural processes, such as volcanism, and anthropogenic processes including coal combustion, mining, manufacturing, and disposal of Hg containing products. Since the start of the industrial era Hg levels in the environment increased dramatically [7, 8] such that anthropogenic sources are now the major contributor to the global Hg budget [9-11]. Due largely to atmospheric emissions, Hg is globally dispersed and high concentrations are often detected in biota of remote areas with no known local inputs [12].

Mercury concentrations in fish are often variable between systems (i.e., lakes) even when these systems are adjacent and apparently have similar inputs [13-15]. This variation is due in part to differences in Hg biogeochemistry and particularly the conversion of inorganic Hg species to MeHg+ and vice-versa, which plays an important role in determining Hg bioavailability, distribution, and toxicity [16-18]. Thus, lake and watershed characteristics that enhance Hg methylation rates, such as the presence of wetlands, the availability of sulfate substrates, and low pH, are widely implicated causes of elevated Hg concentrations in biota [17, 19, 20]. These factors are important in regulating the availability of Hg for uptake at the base of food webs; however, other environmental and ecological properties such as the availability of organic ligands to bind with MeHg+ [21-23], ecosystem productivity [14, 19, 24], community composition [25-28] and food web structure [29-32] also influence Hg cycling through aquatic systems. Further, differences in the age [33], growth rate [34-37], body condition [38, 39], and sex [31, 40-43] of individuals composing populations also contribute to variation in the Hg concentrations of biota.

The structure of food webs is a primary factor regulating Hg accumulation in fishes. Mercury is known to biomagnify through aquatic food webs [44] resulting in increased Hg concentrations in consumers occupying higher trophic positions [TP; i.e., the vertical position within a food web; 30, 45]. Likewise, lakes with more complex food webs containing more trophic interactions (i.e., longer food chains) or increased rates of omnivory [i.e., individuals feeding at more than one trophic level; 46] often have higher Hg concentrations in top consumers [29, 30].

Differential use of resources and variation in underlying dietary pathways (e.g., limnetic vs. benthic feeders) can also result in variable exposure to Hg in consumers [31, 47-49]. However, the role of habitat-specific foraging in determining Hg concentrations in consumers is not consistent across studies and higher Hg concentrations have been measured in both benthic and limnetic consumers. These inconsistencies may arise from differences in Hg bioavailability and concentrations at the bases of benthic and limnetic food webs or due to differences in the transfer of Hg through food webs. Methylmercury is produced most efficiently at the sediment-water interface associated with benthic habitats whereas comparatively little methylation occurs in freshwater limnetic habitats [16], though in some cases limnetic habitats contribute significantly to methylmercury production [50, 51]. Thus, the availability of benthic methylation sites may drive the overall concentration and bioavailability of Hg within a system as consumers more closely aligned with these habitats may experience increased exposure to Hg.

Despite this possibility, Hg concentrations of consumers in many ecosystems are positively correlated with limnetic resource use. A possible explanation for this trend is increased bioconcentration of Hg by limnetic primary producers compared to their benthic counterparts. For example, phytoplankton are known to bioconcentrate Hg at much higher rates than benthic algae [52] and can have Hg concentrations several thousand to millions of times greater than the surrounding water [17, 53, 54].

An additional complication is that the effects of TP and resource use are not strictly independent. Studies in aquatic systems from the tropics to the Arctic have shown that the structure of benthic and limnetic food webs often differs and that these differences

influence the rate and magnitude of Hg biomagnification [29, 31, 55-58]. For example, limnetic food webs are often more complex than benthic food webs and thus individuals or species foraging in the benthic food web typically have lower TPs than those feeding in the limnetic food web [59, 60].

Trophic position and foraging habitat contribute to inter-species [49, 55, 56, 58, 61-63], inter-population [22, 57, 64, 65], and intra-population variation in contaminant concentrations [31, 34, 47, 48, 66]. These effects should be particularly pronounced between benthic and limnetic ecotypes, which are found widely in freshwater fishes such as char (*Salvinus* spp.), whitefish (*Coregonidae*), and stickleback (*Gasterosteidae*).

The threespine stickleback (*Gasterosteus aculeatus*, hereafter stickleback) is a small, phenotypically diverse fish found in marine, brackish, and freshwater environments throughout much of the northern hemisphere. Much of the phenotypic diversity of stickleback is the result of the differentiation of freshwater populations along a continuum between benthic and limnetic ecologies [67]. Benthic and limnetic ecotypes of stickleback occur both allopatrically in thousands of lakes throughout their range, and sympatrically in several lakes along the northern Pacific coast of North America [67-70].

Differences in the morphology, ecology, and life history of benthic and limnetic ecotypes are relatively consistent and well documented in a variety of species [71, 72]. Morphological differences between the ecotypes consist of numerous heritable traits that influence foraging efficiency in the two habitats [67-69, 71, 72]. Across species, benthic fish typically have deeper heads and bodies, fewer more widely spaced gill rakers and more robust cranial morphology, whereas limnetic ecotypes have traits that are well

suited for feeding on planktonic prey including more fusiform body shape, a longer narrower snout, and numerous fine gill rakers [67]. The presence of both sympatric and allopatric ecotypes of stickleback provides an opportunity to contrast the relative importance of ecological factors in determining within- and between-population variation in fish Hg concentrations.

Previously, I documented that Hg concentrations in benthic and limnetic stickleback from Benka Lake in the Cook Inlet basin of Alaska varied with TP and habitat-specific foraging [31]. Specifically, stickleback relying more on limnetic resources had higher mean THg concentrations than individuals utilizing more benthic resources [31]. In the current study I evaluate THg concentrations in stickleback from other Cook Inlet populations (i.e., potentially interbreeding groups of individuals; in the current study population refers to the stickleback from a particular lake) spanning a range of trophic ecologies – including both benthic and limnetic extreme ecotypes as well as populations characterized by more intermediate ecologies – in order to examine whether trophic factors play a similarly significant role in determining inter-lake Hg concentrations. I use stable isotopes of carbon and nitrogen as well as stomach content data to determine TP and the relative reliance on benthic resources (α) and relate these data to variation in THg concentrations both within and between populations. Additionally, I assess whether these relationships vary between sexes both within and among populations. Finally, I relate variation in the THg concentration in stickleback to variation in Hg concentrations at the base of each food web. These data provide a means of assessing the relative importance of trophic factors in determining inter-population variation in Hg accumulation.

3.4 Materials and Methods

3.4.1 Sample collection

Threespine stickleback fish were collected from six lakes in the Cook Inlet Basin from June 7-July 18, 2011 using unbaited 0.64 cm wire mesh minnow traps set near shore. To ensure a range of trophic ecologies were represented in the analyses, dietary and morphological characteristics were used to select lakes representing extreme benthic (Mud and Tern Lakes), intermediate benthic (Corcoran Lake), intermediate limnetic (Stormy Lake), and extreme limnetic (South Rolly and Long Lakes) [67; Figure 3.1]. Fish were euthanized with an overdose of buffered MS-222 anesthetic (Argent Laboratories, Redmond, WA, USA), rinsed in lake water, and stored on crushed dry ice while in the field (2-8 hours), then at -80°C in the laboratory. In order to account for differences in the isotopic baselines of benthic and limnetic food webs, gastropods (*Helisoma anceps* or *Radix auricularia*) and mussels (*Anodonta beringiana*) were collected from the same areas as stickleback and preserved in the same manner. In populations where both species of gastropods were present there were no differences in their isotopic values, therefore both species were utilized to establish isotopic baselines (t = -1.11, df = 14, p = 0.14).

3.4.2 Sample preparation

Analyses initially utilized approximately 30 fish of each sex from each population except South Rolly Lake, from which only 12 individuals were positively identified as female. To minimize errors in sex assignment, only reproductively mature fish (based on gonad

dissections and secondary sexual characteristics) were used. The use of reproductive fish also reduced variation in fish age since most stickleback in these populations spawn at 2 years of age [68, 71, 73]. For each fish, standard length (SL; anterior tip of premaxilla to posterior border of hyperal plate) was measured to the nearest 0.1mm, and a unique specimen identification number was assigned. In addition, stomach contents, the macro-parasite *Schistocephalus solidus* and stickleback eggs were removed to prevent biases due to the presence of unassimilated materials and the high lipid content of eggs (mean lipid content of stickleback eggs = 10.5% dry weight [dw], n = 36). The shell and gut contents of gastropod and mussel samples were also removed prior to preparation for analyses.

Eviscerated stickleback carcasses were freeze-dried for 72 hr. and then ground into a fine powder using a cryogenic tissue mill (SPEX SamplePrep, Metuchen, NJ, USA). Gastropod and mussel samples were freeze-dried for 72 hr. (gastropods) or 144 hr. (mussels; due to their larger volumes) and then homogenized in the tissue mill.

3.4.3 Stable isotope analysis

For each sample, approximately 1.0 ± 0.2 mg of dried homogenate was sealed in a tin capsule and analyzed for stable carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) isotope ratios at the University of Alaska Anchorage – Environmental and Natural Resources Institute Stable Isotope Laboratory using a Thermo-Finnigan Delta Plus XP Isotope Ratio Mass Spectrometer (IRMS) coupled to a Costech 4010 Elemental Combustion System. Isotope ratios are presented in standard δ notation as parts per thousand (‰) differences between

the isotope ratio of the sample and that of a standard (Vienna Pee Dee Belemnite for carbon and air for nitrogen) using the formula:

$$\delta R = [(R_{sample} - R_{standard}) / R_{standard}] \times 1000$$

where R is the carbon ($^{13}C/^{12}C$) or nitrogen ($^{15}N/^{14}N$) isotope ratio of the sample or the standard. Samples depleted in the heavier isotope (^{13}C or ^{15}N) in comparison to the standard have lower delta values. All instruments are regularly calibrated using a suite of International Atomic Energy Agency standards to ensure instrument precision, accuracy, and tuning parameters are to specification. At least six replicates each of a stable isotope reference material (SRM 1547: peach leaves, available from the National Institute of Standards and Technology; $\delta^{13}C = -25.89 \pm 0.3‰$, $\delta^{15}N = 1.90 \pm 0.3‰$) and laboratory working standard (Cheney Lake stickleback; $\delta^{13}C = -29.48 \pm 0.2‰$, $\delta^{15}N = 9.77 \pm 0.1‰$) were analyzed with every batch of 40 samples. External instrument reproducibility for carbon isotope analysis was ± 0.2‰ and ± 0.1‰ for nitrogen analysis. Isotope values were not lipid normalized because lipid content is uniformly low in stickleback carcasses (mean percent lipid of males and females with eggs removed = 2.1 ± 0.7%; C:N = 4.14 ± 0.8, n = 340).

3.4.4 Mercury analysis

Mercury concentrations were determined for all stickleback, bivalves and gastropods at the U.S. Geological Survey, Forest and Rangelands Ecosystem Science Center in Corvallis, OR via thermal decomposition and cold-vapor atomic absorption spectroscopy using a DMA-80 Direct Mercury Analyzer (Milestone Inc., Shelton, CT, USA) and following EPA method 7473 [74]. Quality assurance measures included

analysis of two certified reference materials (CRM; dogfish muscle tissue [DORM-4], 0.41 μg/g dw, and lobster hepatopancreas [TORT-2], 0.27 μg/g dw; National Research Council of Canada, Ottawa, Canada), system blanks, method blanks, calibration standards and two duplicates per batch of 40 samples. Recoveries of THg averaged 104.2 ± 1.4% (*n* = 23) for CRMs and 99.5 ± 0.8% (*n* = 34) for calibration standards. Relative percent difference (RPD) for all duplicates averaged 7.6 ± 1.5% (n = 22). All THg data are presented on a dry weight basis in order to minimize variance due to variable moisture contents; however, concentrations can be converted to wet weight estimates using the mean percent moisture of stickleback used in this study (78.3% ± 0.03%, n = 339).

3.4.5 Statistical and stomach contents analysis

In order to assess the importance of trophic ecology as a determinant of THg concentration, I identified stomach contents for all fish to family or genus, and classified each prey taxa as benthic or limnetic based on ecological descriptions [75-77]. The proportion of benthic prey (α) in each stomach was then calculated by dividing the mass of benthic prey items by the mass of all prey items. These data were coupled with stickleback nitrogen stable isotope data to calculate trophic position (TP) of individual stickleback following Post [45].

Mercury concentrations in fish are influenced by Hg bioavailability at the base of the foodweb as well as processes that influence the propagation of Hg through the foodweb [44]. In order to differentiate between these two processes, I controlled for variation in Hg bioavailability at the base of the foodwebs in the six lakes by normalizing

Hg values by the Hg values in primary consumers (bivalves and gastropods) using the formula:

$$THg_{norm\ i} = THg_{fish\ i} - ([THg_{benthic} \times \alpha_{fish\ i}] + ([THg_{limnetic} \times (1 - \alpha_{fish\ i})])$$

where $THg_{norm\ i}$ is the normalized THg concentration of fish *i*, $THg_{fish\ i}$ is the non-normalized THg concentration of fish *i*, $THg_{benthic}$ is the average THg concentration in gastropods from the lake, $THg_{limnetic}$ is the average THg concentration in bivalves from the lake, and $\alpha_{fish\ i}$ is the proportion of the diet of fish *i* that is derived from benthic sources as estimated via stomach contents analysis. These "baseline-normalized" THg values provide a means of distinguishing between variation in trophic processes (i.e., the movement of Hg through foodwebs) as opposed to habitat-specific availability of Hg (i.e., the production of MeHg+ and its uptake in primary consumers). Additionally, I calculated size-corrected, baseline-normalized Hg values by regressing SL against THg_{norm} in each lake and then adding the resulting residuals to the THg_{norm} value estimated at the mean SL across populations ($\bar{x}$ = 47.27 mm, n = 291). The same process was used to size-correct the raw (i.e., non-normalized) THg concentrations.

Prior to analysis, size-corrected THg concentrations and THg_{norm} values were natural-log transformed and dietary proportions determined from stomach content analysis were arcsine square-root transformed [78] in order to meet the assumptions for parametric modeling. Fish with empty stomachs (n = 48) were excluded, leaving 291 fish in the modeling analyses.

Multiple regression coupled with quantitative model selection techniques were used to examine the effects of sex, α, and TP on size-corrected fish THg concentrations (non-

baseline normalized). Sample size corrected Akaike's Information Criterion (AIC_C) was used to select the most parsimonious model for explaining THg concentrations from an initial set of 47 *a priori* candidate models that included all possible main-effects combinations, as well as interactions between covariates and a null (intercept only) model (Table 3.S1). I ranked the candidate models using the AIC_C differences between the best model and the other candidate models [ΔAIC_C; 79]. The Akaike weights (w_i) of candidate models were compared to assess each model's probability of being the best fitting model and to calculate each parameter's variable weight (V), a measure of the relative importance of a parameter across models [79]. In addition, I calculated model-averaged beta-coefficients for each parameter using the full set of all candidate models. Model-averaged estimates provide a more robust representation of the "true" relationship between a parameter and THg concentrations across the range of model possibilities observed [79].

Using the global models I was unable to examine the importance of sex, α, and TP within populations due to interactions between population and the covariates. Therefore, a set of 13 candidate models were compared for each population (Table 3.S2) and assessed in the same manner as described above.

Finally, to examine the influence of inter-lake variation on the bioavailability of Hg at the base of foodwebs, the global model analyses were repeated using size-corrected, baseline-normalized Hg values (THg_{norm}; Table 3.S3). Differences in the model averaged beta-coefficients and variable weights derived from the models using THg_{norm} were then compared to those derived from non-normalized Hg concentrations.

All analyses were conducted in R version 2.15.0 [80] and data are presented as back-transformed least-square means from the model outputs with standard errors.

3.5 Results

My data indicate that stickleback from the six study lakes differed in numerous aspects of their biology. The SL of stickleback ranged from 37.4 to 62.8 mm with significant differences among lake means ($F_{5,285} = 18.39$, $p < 0.001$; Table 3.1). Mean reliance on benthic prey (α) based on stomach contents varied between lakes ($F_{5,285} = 31.92$, $p < 0.001$) and ranged from 0.25 ± 0.3 for South Rolly Lake to 0.92 ± 0.2 for Mud Lake. Mean TP ranged from 3.30 ± 0.3 in Long Lake to 3.96 ± 0.2 in Tern Lake and also differed among lakes ($F_{5,285} = 33.02$, $p < 0.001$).

3.5.1 Non-baseline normalized THg

There were also numerous differences in THg levels in the six study lakes. Mean THg concentrations were significantly different among lakes for bivalves ($F_{5,48} = 6.24$, $p < 0.001$) and stickleback ($F_{5,285} = 76.91$, $p < 0.001$), but not gastropods ($F_{5,48} = 1.25$, $p = 0.301$). Stickleback THg was lowest in Long Lake (112 ± 7 ng/g dw) and highest in Mud Lake (344 ± 13 ng/g dw; Table 1). Across populations, when all fish were included in a single analysis, there was a positive correlation between size-corrected THg concentration and α ($r = 0.23$, $DF = 289$, $p < 0.001$). Population mean THg concentrations were also correlated with mean α values across lakes (Spearman rank correlation rho (ρ) = 0.82, $p = 0.003$), though within lakes there was generally no significant relationship. Population mean THg concentrations were not correlated with

mean TP across lakes ($\rho = 0.60$, $p = 0.24$), but there was a correlation within lakes ($r = 0.143$, DF = 289, $p < 0.015$; Fig. 3.2).

After controlling for the effects of sex, α, and TP, lake-specific mean THg concentrations of stickleback ranged from 126 ± 13 ng/g dw in Stormy Lake to 363 ± 15 ng/g dw in Mud Lake. Across populations female stickleback had a higher mean THg concentration ($\bar{x}$ = 221 ± 12 ng/g dw) than males ($\bar{x}$ = 195 ± 10 ng/g dw; $t = 2.34$, df = 289, $p = 0.01$) when the effects of α and TP were controlled for (Fig. 3.3). In five of the populations THg concentrations increased by approximately 3-fold over the nearly 2-fold range (2.57 to 5.05) of observed TPs (Fig. 3.4) after controlling for the effects of sex and α. In the remaining population (Stormy Lake), THg concentrations in stickleback decrease by approximately 1.8-fold over the observed range of TP (Fig. 3.4). There was no relationship between THg concentrations and α either within or among lakes after controlling for the effects of sex and TP.

Using quantitative model selection I found that the most parsimonious model explaining size-corrected THg concentrations across lakes included sex, population, and TP as well as a population by TP interaction ($w_i = 0.30$; Table 3.S1). This model was 1.5 times more likely than the next most plausible model ($\Delta AIC_c = 0.86$) which was identical to the top model but without the population by TP interaction. Variable weights indicated that TP and sex were both important determinants of THg concentrations across the model sets ($V_{TP} = 0.99$, $V_{sex} = 0.98$) and that α was less important ($V_{\alpha} = 0.28$; Table 3.2).

The most parsimonious models explaining THg concentrations within a population varied and in some cases the top models had little support (Table 3.S2). Therefore, I used model averaging to generate coefficient estimates that incorporate model selection uncertainty. There was also substantial variability in the relative importance and directional effect of individual parameters. Sex and TP were on average the most important parameters (mean $V_{sex} = 0.75 \pm 0.31$, mean $V_{TP} = 0.73 \pm 0.28$) but both had variable weights ranging from approximately 0.35 to 1.00 depending on population (Table 3.2). Reliance on benthic prey (α) was on average less important than either sex or TP (mean $V_{\alpha} = 0.39 \pm 0.19$); however, α also varied in importance and in Long Lake had a variable weight similar to sex and TP.

3.5.2 Baseline-normalized THg

After correcting for the effects of size and normalizing by Hg levels in primary consumers, Hg_{norm} values of stickleback differed among lakes ($F_{5,285} = 33.03$, $p < 0.001$), ranging from 39 ± 4 ng/g dw in Stormy Lake to 233 ± 13 ng/g dw in Mud Lake. Across populations the Hg_{norm} values of individuals were not correlated with either α ($r = 0.088$, $df = 289$, $p = 0.142$) or TP ($r = -0.005$, $df = 289$, $p = 0.938$). Similarly, population mean Hg_{norm} values were not correlated with α ($\rho = 0.26$, $p = 0.658$) or TP ($\rho = 0.09$, $p = 0.919$).

The top model explaining Hg_{norm} values across lakes included only sex and population. The models including sex and population along with either α or TP were also plausible ($\Delta AIC_c = 1.19$ and 1.25, respectively); however, the top model was 1.8 times more likely ($w_i = 0.27$) than either of these alternatives ($w_i = 0.15$ for both; Table 3.S3).

As in the models of non-baseline-normalized THg concentrations sex was an important variable (V_{sex} = 0.98); however, TP was clearly less important than sex in the Hg_{norm} models (V_{TP} = 0.52). The relative importance of α (V_{α} = 0.43) was again less than that of TP, but the difference was comparatively small in the Hg_{norm} models (Table 3.2).

3.6 Discussion

Despite the lack of any known local anthropogenic sources of Hg, I found substantial variation in THg concentrations of stickleback from the six lakes that I sampled. Across the lakes THg concentrations varied by approximately 13.5-fold, with concentrations in individual lakes spanning on average a 4.4-fold and up to 5.3-fold range. I utilized this variation to evaluate the roles of sex and ecological factors, namely foraging habitat and trophic position, in determining THg concentrations both within and among populations.

Mercury accumulation in fishes is influenced by numerous factors acting at multiple hierarchical levels [81]. The variable importance of these factors, and complex interactions between factors operating at different levels, account for the extensive spatial variability in fish Hg concentrations even in geographically adjacent populations [13, 14]. The results of the current study indicate that while sex, foraging habitat, and TP are important determinants of THg concentrations in some stickleback populations, their roles are inconsistent across populations and likely confounding.

In the current study I found that THg concentrations in stickleback were positively correlated with α values of all individuals pooled across all populations and of population

mean α values. While similar trends have been observed in some studies [47, 62, 63], the majority have found that fish foraging on limnetic prey have higher Hg concentrations than those foraging on benthic prey [49, 55-58, 64, 65, 82]; indeed, I also found this to be the case in Benka Lake, another population in my study system [31]. This pattern of elevated Hg in limnetic food webs has been widely explained by differences in the assimilation of Hg at the base of food webs; limnetic phytoplankton are known to bioconcentrate Hg to much higher levels than benthic algae [83], and biological characteristics (e.g., trophic position, age, energetics, etc.) may favor accumulation in limnetic food webs. Where higher Hg concentrations have been observed in benthic foraging individuals, such as in the current study, increased production of bioavailable MeHg+ in benthic habitats has been cited as the likely cause [47, 62, 63]; however, to my knowledge there has been little data to directly support this mechanism.

The results of the present study provide support for the hypothesis of increased bioavailability of Hg in lakes dominated by benthic habitat by demonstrating that the correlation between fish THg concentration and α is absent when Hg concentrations are normalized for inter-lake variation in Hg bioavailability at low trophic positions. These data suggest that the net effects of habitat-specific processes on fish Hg concentrations is a balance between the processes of MeHg+ production in benthic sediments, which leads to higher MeHg+ levels in benthic habitats, and the increased bioconcentration of this bioavailable Hg into limnetic food webs. Since the pool of bioavailable Hg within the surface waters of a lake is relatively well-mixed [i.e., bioavailable Hg is dispersed through all surficial habitats; 84-87], MeHg+ availability (i.e., production and inputs) is

likely the primary driver of inter-population variation in fish Hg concentrations while habitat-specific foraging is more likely to play a role in determining within population patterns of Hg concentrations. This conclusion is supported by a large number of studies that have found lake-level physical and chemical characteristics, particularly those impacting lake methylation potential, to correlate more closely with fish Hg concentrations than biological characteristics of the fish [57, 64, 65, 88, 89].

Across populations, sex had the most consistent effect on THg concentrations with female fish having higher mean concentrations than males after accounting for α and TP. These results are in contrast to previous work with stickleback from Benka Lake [31], also located in the Cook Inlet Basin, and lakes on Agattu Island in the Aleutian Archipelago of Alaska (Kenney *unpublished data*), in which female stickleback have been shown to have lower mean THg concentrations than males. The results of the current study also contrast with an extensive body of literature on other species of fish [33, 40, 42, 43, 90-94]. However, Shedd [82] found that female stickleback from Jo-Jo Lake in southwest Alaska had consistently higher THg concentrations than males despite having lower TPs and higher α values.

The seemingly contradictory nature of these results when taken as a whole suggest that the relationship between sex and Hg accumulation in stickleback is complex and likely reflects the integration of numerous differences in the ecology and physiology of the sexes. Many populations of stickleback display sexual differentiation in habitat use and specialization on specific prey within habitats, though the degree and direction of this differentiation is also variable across populations and regions [95-98]. Further, sexual

dimorphism in stickleback populations appears to vary both with the trophic habits and niche breadth of the population as a whole, suggesting that the relationship between females and males is different depending on the ecology of lakes [95, 99]. Similarly, Stacy and Lepak [94] suggest that differences in the Hg concentrations of female and male walleye (*Sander vitreus*) may be due to sexually divergent exploitation of specific food resources that are dependent on food-web specific characteristics. While my data do not discount the ecology of the sexes as a driver of differences in their THg concentrations, the persistence and even strengthening of the differences when α and TP are accounted for suggest that other parameters are more important than trophic ecology.

My results indicate that there is substantial inter-population variability in the importance of TP and α in determining THg concentrations of stickleback. Similarly, the fishes of Jo-Jo Lake in southwest Alaska display variable importance of TP and α depending upon species, sex, and size-class specific interactions [82]. Willacker et al. [31] also found sex-specific differences in the relative importance of α and TP in determining THg concentrations of stickleback.

The observed variation in the importance of TP and α likely arises from standing variation in the specialization of individuals within each population. Many species exhibit substantial variation in individual utilization of available resources [i.e., individual niche variation; 100], which in turn structures population level niche variation, an important ecological attribute of populations that has the potential to alter ecological functioning in numerous ways [101-103]. Recent studies to examine the underlying mechanisms controlling the magnitude and strength (i.e., resistance to changes) of niche

variation indicate that there are few processes consistent across systems. Rather, niche width variation appears to depend on complex interactions between individual and ecosystem attributes including resource availability, abundance and diversity [104, 105], population and community structure [106], phenotypic variability [102, 107, 108], physiological demands of individuals [109, 110], competition [111-113], predation regimes [114, 115] and a multitude of other factors [116]. This tremendous variation in the mechanisms generating dietary variation, and the multitude of potential feedbacks associated with these processes, may preclude consistent relationships between the trophic characteristics of individuals and contaminant accumulation at levels beyond that of the population.

While considerable variation in the importance of α and TP exists, a growing body of literature indicates that when both variables are considered concurrently, TP is typically more important in determining THg concentrations than α [31, 62, 82, 117-119]. In the present study, I found that after controlling for sex and TP there was no residual relationship between THg concentrations and α in any of the populations. In contrast, a robust relationship remained between TP and THg concentrations both within and among populations after controlling for variations in sex and α. This result suggests that TP influences THg concentrations independent of other trophic processes, whereas the effects of habitat-specific foraging may represent correlated effects or be confounded by other variables. However, it is notable that when differences in THg concentrations at low trophic levels were accounted for the importance of TP declined from 0.99 to 0.52, whereas that of α increased from 0.28 to 0.43, suggesting that the relationship between

TP and fish THg concentrations is influenced by habitat-specific processes and that the importance of TP may be overestimated when these processes are ignored.

I sampled stickleback from six lake populations spanning a range of trophic ecologies to determine the relative importance of sex, trophic position, and reliance on benthic prey in determining THg concentrations. My data suggest that across populations sex and trophic position are more important than reliance on benthic prey under these study conditions; however, there was substantial variation in the relative importance of these parameters in individual lakes. This inter-population variation indicates that the mechanisms underlying the observed patterns in THg concentrations are poorly understood and should be further investigated. Across lakes I found a positive correlation between THg concentrations in stickleback and the reliance on benthic prey, a result that is in contrast with other studies examining the role of habitat-specific foraging in stickleback and other species [31, 49, 55-57, 64, 65, 82, 120]. However, when inter-population variation in primary consumer THg concentrations was accounted for this relationship no longer existed, suggesting that differences in Hg bioavailability and concentrations at the base of the food webs were the primary driver of variation in THg concentrations in sticklebacks across lakes. Thus, further research aimed at understanding Hg concentrations in fish should focus on lake and landscape scale factors that influence the production of methylmercury. These data contribute to our understanding of the mechanisms by which intra- and inter-population variation in THg concentrations of lower trophic level fish arise and thus provide important insights into

the processes regulating Hg transfer to higher trophic level consumers such as piscivorous fish, birds, and mammals.

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3.9 Tables

Table 3.1: Mean ± standard error for variables measured in stickleback from six study lakes in the Cook Inlet Basin, Alaska. Geometric means are presented for mercury concentrations and arithmetic means for the remaining variables.

Lake	Ecotype	N	Standard length (mm)	$\delta^{13}C$ (‰)	α[a]	$\delta^{14}N$ (‰)	TP[b]	THg[c] (ng g^{-1} dw)	THg_{norm}[d] (ng g^{-1} dw)
Corcoran	Intermediate-benthic	47	50.4 ±5.0	-26.5 ±1.5	0.85 ±0.2	9.5 ±1.0	3.72 ±0.4	209 ±12	79 ±12
Long	Limnetic	49	42.9 ±3.4	-30.2 ±1.6	0.47 ±0.4	8.4 ±0.8	3.30 ±0.3	112 ±7	47 ±7
Mud	Benthic	56	47.7 ±3.7	-25.2 ±1.3	0.92 ±0.2	8.6 ±0.6	3.37 ±0.2	344 ±13	233 ±13
South Rolly	Limnetic	35	45.5 ±6.1	-29.7 ±2.1	0.25 ±0.3	9.3 ±1.5	3.65 ±0.6	176 ±13	72 ±13
Stormy	Intermediate	51	49.7 ±4.6	-23.9 ±1.8	0.71 ±0.4	8.5 ±0.7	3.33 ±0.3	123 ±3	40 ±3
Tern	Benthic	53	46.9 ±5.1	-29.2 ±1.3	0.80 ±0.3	10.1 ±0.6	3.96 ±0.2	202 ±9	77 ±9

[a] The proportion of the diet from benthic sources; [b] trophic position; [c] size-corrected total mercury concentration; [d] size-corrected, baseline-normalized total mercury concentration (see text for derivation).

Table 3.2: Relative importance of sex, trophic position (TP), and reliance on benthic prey (α) in determining total mercury concentrations in stickleback from six lakes in the Cook Inlet Basin, Alaska. Variable weights were calculated as the sum of Akaike weights for all models containing each variable in three sets of models: global models (all lakes) with non-baseline corrected total mercury concentrations, population specific models with non-baseline corrected total mercury concentrations, and global models with baseline corrected total mercury concentrations.

Model Set	Sex	TP	α
Non-normalized			
Global	0.98	0.99	0.28
Populations			
Corcoran	1.00	0.98	0.50
Long	1.00	1.00	0.91
Mud	0.86	0.96	0.36
South Rolly	0.92	1.00	0.24
Stormy	0.36	0.96	0.28
Tern	0.34	0.36	0.27
Baseline-normalized			
Global	0.98	0.52	0.43

3.10 Figures

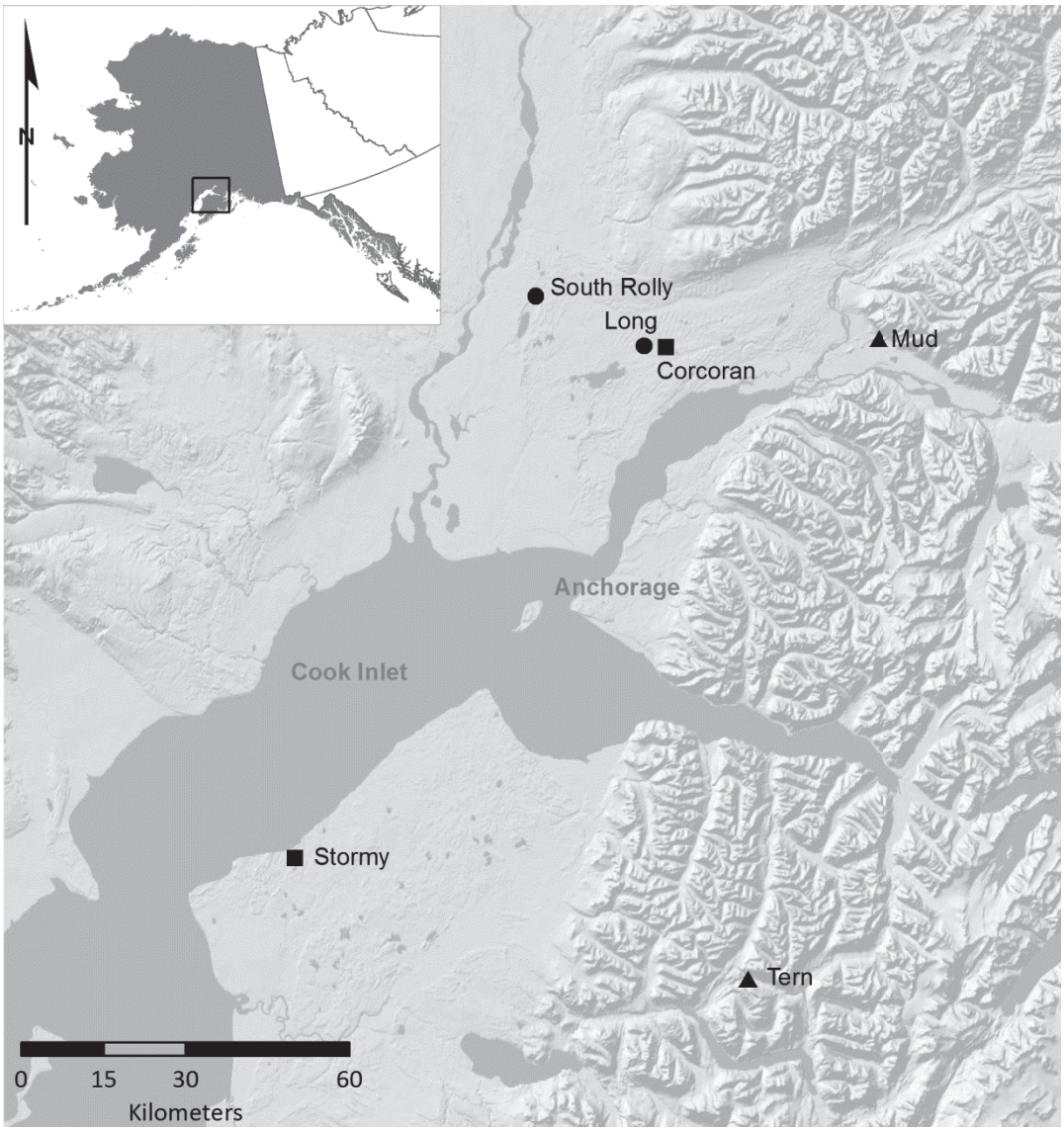

Figure 3.1: Locations of six study lakes within the Cook Inlet Basin of Alaska. Triangles denote lakes with extreme benthic ecotypes, squares indicate intermediate ecotypes, and circles indicate extreme limnetic ecotypes.

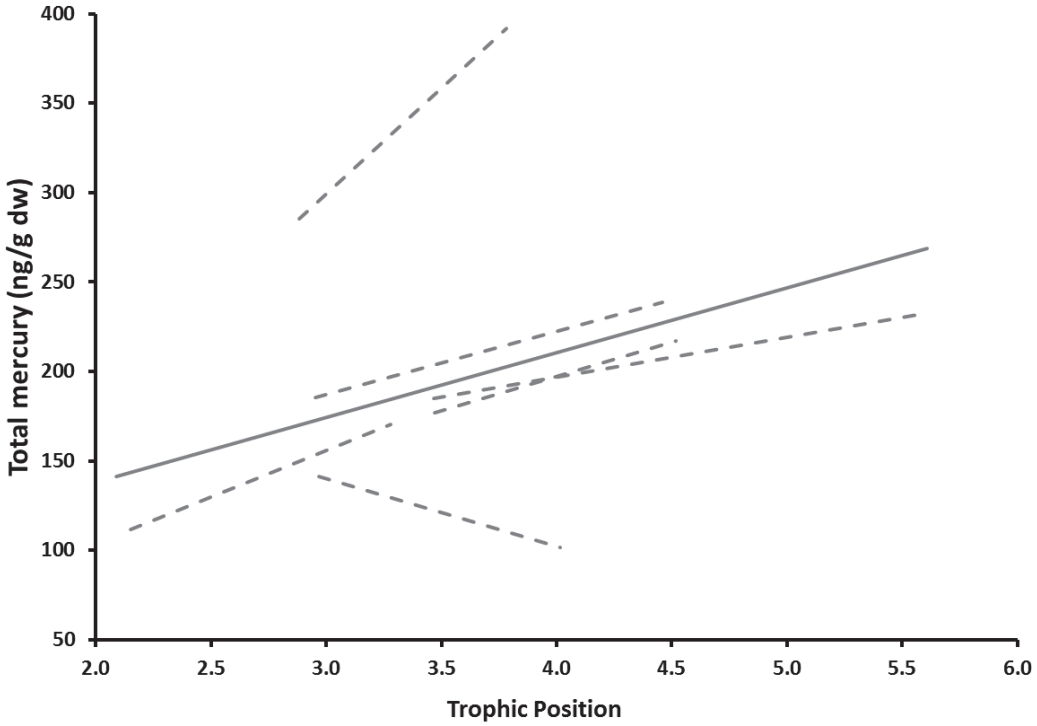

Figure 3.2: Relationships between total mercury concentration and trophic position in stickleback from six lakes (dashed lines) in the Cook Inlet Basin of Alaska and all lakes combined (solid line).

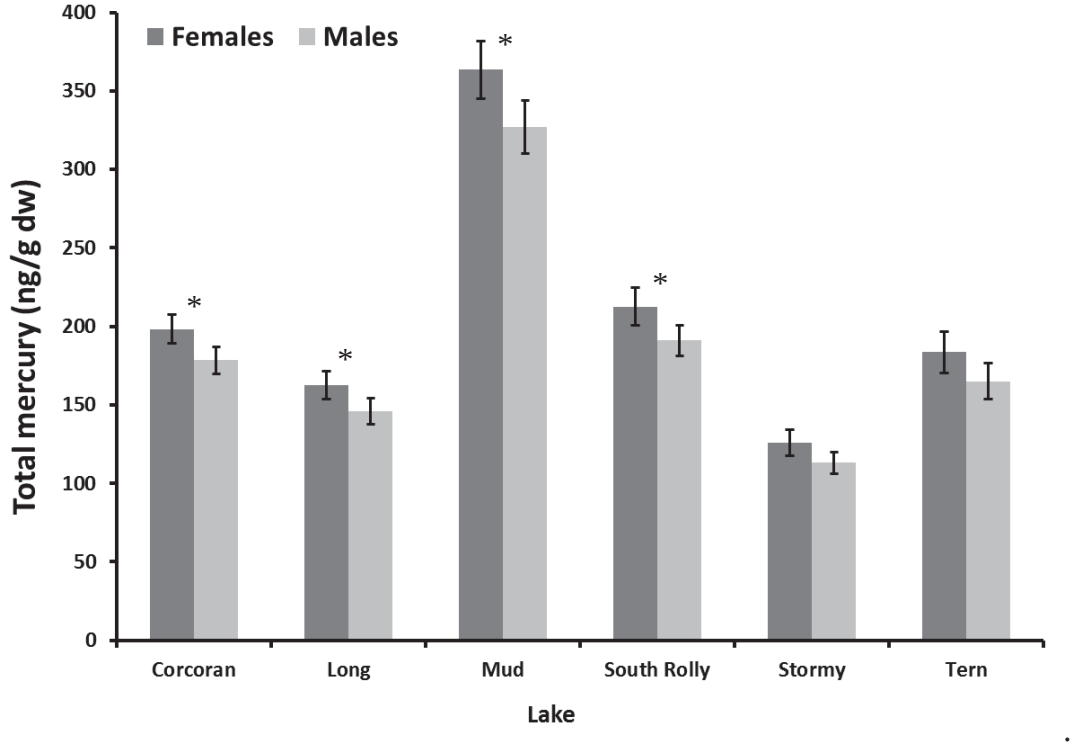

Figure 3.3: Back transformed least-square mean total mercury concentrations in stickleback from six study lakes in the Cook Inlet Basin of Alaska. Error bars represent standard error and asterisks indicate populations with significant differences between female and male concentrations.

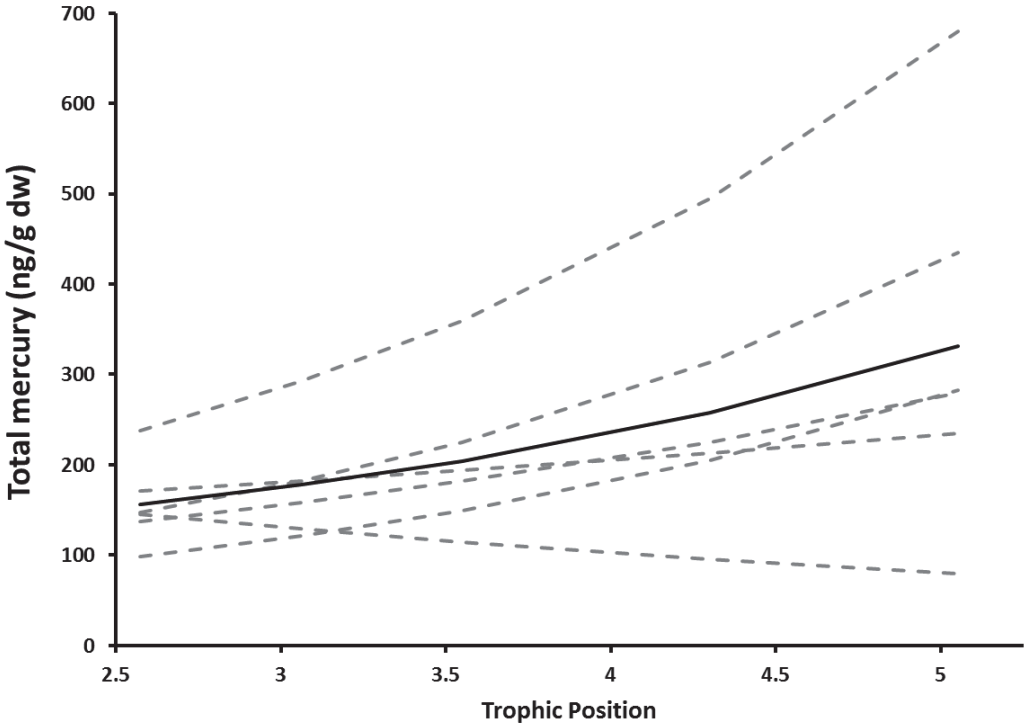

Figure 3.4: Model averaged estimates of total mercury concentrations across the range of observed trophic positions in stickleback from six lakes (dashed lines) in the Cook Inlet Basin of Alaska and all lakes combined (solid line).

3.11 Supplemental Information

Table 3.S1: Ranking criteria and the structure of global candidate models describing non-baseline-normalized total mercury concentrations in stickleback from six study lakes in the Cook Inlet Basin, Alaska. All models are based on a sample size of 291 fish and include additive (+) and interaction (*) terms.

Model Structure[a]	R^2	k[b]	LogL[c]	AIC_C[d]	ΔAIC_C	w_i[f]
Sex+Pop+TP+(Pop*TP)	0.60	14	-34.25	98.03	0.00	0.30
Sex+Pop+TP	0.59	9	-40.12	98.89	0.86	0.19
Sex+Pop+TP+(Sex*TP)	0.59	10	-39.62	100.02	2.00	0.11
Sex+Pop+α+TP+(Pop*TP)	0.59	15	-34.25	100.24	2.21	0.10
Sex+Pop+TP+(Sex*TP)+(Pop*TP)	0.59	15	-34.25	100.24	2.22	0.10
Sex+Pop+α+TP	0.58	10	-40.09	100.96	2.94	0.07
Sex+Pop+α+TP+(Sex*TP)	0.58	11	-39.59	102.12	4.10	0.04
Sex+Pop+α+TP+(Sex*TP)+(Pop*TP)	0.59	16	-34.24	102.47	4.44	0.03
Sex+Pop+α+TP+(Sex*α)+(Pop*TP)	0.59	17	-34.00	104.24	6.22	0.01
Pop+TP+(Pop*TP)	0.58	13	-38.62	104.56	6.53	0.01
Sex+Pop+α+TP+(Sex*α)	0.58	12	-39.94	105.00	6.98	0.01
Sex+Pop	0.58	8	-44.28	105.07	7.04	0.01
Sex+Pop+α+TP+(Sex*α)+(Sex*TP)	0.58	13	-39.38	106.08	8.05	0.01
Sex+Pop+α+TP+(Sex*α)+(Sex*TP)+(Pop*TP)	0.59	18	-33.99	106.49	8.47	0.00
Pop+α+TP+(Pop*TP)	0.58	14	-38.62	106.77	8.74	0.00
Sex+Pop+α	0.57	9	-44.26	107.17	9.14	0.00
Pop+TP	0.57	8	-45.81	108.13	10.10	0.00
Pop+α+TP	0.57	9	-45.66	109.95	11.93	0.00
Sex+Pop+α+(Sex*α)	0.57	11	-43.51	109.97	11.94	0.00
Sex+Pop+α+TP+(Pop*α)	0.58	16	-38.47	110.92	12.89	0.00
Sex+Pop+α+TP+(Pop*α)+(Pop*TP)	0.59	21	-33.11	111.65	13.63	0.00
Sex+Pop+α+TP+(Sex*TP)+(Pop*α)	0.58	17	-38.06	112.37	14.34	0.00
Sex+Pop+α+TP+(Sex*α)+(Pop*α)	0.58	17	-38.34	112.92	14.89	0.00
Sex+Pop+α+TP+(Sex*α)+(Pop*α)+(Pop*TP)	0.59	22	-32.86	113.49	15.47	0.00
Pop	0.56	7	-49.69	113.77	15.74	0.00
Sex+Pop+α+TP+(Sex*TP)+(Pop*α)+(Pop*TP)	0.59	22	-33.11	113.99	15.97	0.00
Sex+Pop+α+TP+(Sex*α)+(Sex*TP)+(Pop*α)	0.58	18	-37.90	114.32	16.29	0.00
Pop+α	0.56	8	-49.59	115.68	17.66	0.00
Sex+Pop+α+TP+(Sex*α)+(Sex*TP)+(Pop*α)+(Pop*TP)	0.59	23	-32.86	115.85	17.82	0.00
Sex+Pop+α+(Pop*α)	0.57	15	-42.65	117.05	19.03	0.00
Sex+Pop+α+(Sex*α)+(Pop*α)	0.57	16	-41.88	117.74	19.71	0.00
Pop+α+TP+(Pop*α)+(Pop*TP)	0.58	20	-37.60	118.32	20.29	0.00
Pop+α+TP+(Pop*α)	0.56	15	-44.26	120.27	22.24	0.00
Pop+α+(Pop*α)	0.55	14	-48.16	125.84	27.81	0.00
Sex+α+TP+(Sex*α)	0.09	7	-156.23	326.86	228.84	0.00
Sex+α+TP	0.08	5	-158.58	327.37	229.35	0.00
Sex+α+TP+(Sex*α)+(Sex*TP)	0.08	8	-156.21	328.93	230.90	0.00
Sex+α+TP+(Sex*TP)	0.08	6	-158.46	329.22	231.19	0.00
α+TP	0.07	4	-160.84	329.82	231.79	0.00
Sex+α+(Sex*α)	0.07	6	-159.37	331.03	233.00	0.00
Sex+α	0.06	4	-161.66	331.47	233.44	0.00
α	0.05	3	-163.74	333.56	235.53	0.00
Sex+TP	0.04	4	-165.44	339.03	241.00	0.00
Sex+TP+(Sex*TP)	0.03	5	-165.43	341.08	243.05	0.00

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Model Structure[a]	R^2	k[b]	LogL[c]	AIC_C[d]	ΔAIC_C	w_i[f]
Sex	0.02	3	-168.66	343.40	245.38	0.00
TP	0.02	3	-168.91	343.90	245.87	0.00
Null	0.00	2	-171.90	347.84	249.81	0.00

[a] Sex = sex, Pop = population, α = percent reliance on benthic prey, and TP = trophic position; [b] The number of estimated parameters in the model including the intercept and variance; [c] Log-likelihood of the regression model; [d] Sample size corrected Akaike's Information Criterion; [e] The difference between the current model AIC_C and the AIC_C of the most parsimonious model; [f] Akaike's weight; the likelihood of the current model relative to others in the candidate set.

Table 3.S2: Ranking criteria and the structure of candidate models describing non-baseline-normalized total mercury concentrations in Corcoran Lake stickleback. All models are based on a sample size of 47 fish and include additive (+) and interaction (*) terms.

Model Structure[a]	R^2	k[b]	LogL[c]	AIC_C[d]	ΔAIC_C	w_i[f]
Sex+TP	0.34	4	-9.20	27.34	0.00	0.34
Sex+α+TP	0.34	5	-8.52	28.51	1.17	0.19
Sex+α+TP+(Sex*α)	0.36	6	-7.29	28.69	1.35	0.18
Sex+TP+(Sex*TP)	0.34	5	-8.83	29.12	1.78	0.14
Sex+α+TP+(Sex*TP)	0.34	6	-8.27	30.63	3.29	0.07
Sex+α+TP+(Sex*α)+(Sex*TP)	0.36	7	-7.00	30.88	3.54	0.06
Sex	0.22	3	-13.63	33.82	6.48	0.01
Sex+α	0.22	4	-13.20	35.36	8.02	0.01
Sex+α+(Sex*α)	0.22	5	-12.72	36.91	9.57	0.00
Null	0.00	2	-20.03	44.34	16.99	0.00
TP	0.01	3	-19.16	44.88	17.54	0.00
A	-0.02	3	-19.94	46.45	19.10	0.00
α+TP	0.00	4	-19.07	47.09	19.75	0.00

[a] Sex = sex, Pop = population, α = percent reliance on benthic prey, and TP = trophic position; [b] The number of estimated parameters in the model including the intercept and variance; [c] Log-likelihood of the regression model; [d] Sample size corrected Akaike's Information Criterion; [e] The difference between the current model AIC_C and the AIC_C of the most parsimonious model; [f] Akaike's weight; the likelihood of the current model relative to others in the candidate set.

Table 3.S3: Ranking criteria and the structure of candidate models describing non-baseline-normalized total mercury concentrations in Long Lake stickleback. All models are based on a sample size of 49 fish and include additive (+) and interaction (*) terms.

Model Structure[a]	R^2	k[b]	LogL[c]	AIC_C[d]	ΔAIC_C	w_i[f]
Sex+α+TP+(Sex*TP)	0.53	6	-5.91	25.81	0.00	0.51
Sex+α+TP+(Sex*α)+(Sex*TP)	0.54	7	-4.80	26.32	0.51	0.40
Sex+TP+(Sex*TP)	0.48	5	-8.96	29.31	3.50	0.09
Sex+TP	0.37	4	-14.52	37.94	12.13	0.00
TP	0.34	3	-16.01	38.55	12.74	0.00
Sex+α+TP	0.37	5	-13.65	38.69	12.88	0.00
α+TP	0.35	4	-14.92	38.75	12.94	0.00
Sex+α+TP+(Sex*α)	0.38	6	-12.92	39.84	14.03	0.00
Sex+α+(Sex*α)	0.16	5	-20.92	53.24	27.43	0.00
Sex+α	0.10	4	-23.09	55.09	29.28	0.00
Sex	0.07	3	-24.34	55.20	29.39	0.00
A	0.04	3	-25.08	56.70	30.89	0.00
Null	0.00	2	-26.70	57.67	31.86	0.00

[a] Sex = sex, Pop = population, α = percent reliance on benthic prey, and TP = trophic position; [b] The number of estimated parameters in the model including the intercept and variance; [c] Log-likelihood of the regression model; [d] Sample size corrected Akaike's Information Criterion; [e] The difference between the current model AIC_C and the AIC_C of the most parsimonious model; [f] Akaike's weight; the likelihood of the current model relative to others in the candidate set.

Table 3.S4: Ranking criteria and the structure of candidate models describing non-baseline-normalized total mercury concentrations in Mud Lake stickleback. All models are based on a sample size of 56 fish and include additive (+) and interaction (*) terms.

Model Structure[a]	R^2	k[b]	LogL[c]	AIC_C[d]	ΔAIC_C	w_i[f]
Sex	0.16	4	-2.84	14.47	0.00	0.35
α	0.16	5	-2.30	15.80	1.33	0.18
TP	0.15	5	-2.49	16.17	1.70	0.15
Sex+α	0.09	3	-5.44	17.35	2.87	0.08
Sex+TP	0.15	6	-1.89	17.49	3.02	0.08
α+TP	0.14	6	-2.37	18.45	3.97	0.05
Sex+α+TP	0.09	4	-5.02	18.82	4.35	0.04
Null	0.14	7	-1.78	19.89	5.42	0.02
Sex+α+TP+(Sex*α)	0.04	3	-7.02	20.50	6.03	0.02
Sex+α+TP+(Sex*TP)	0.00	2	-8.61	21.44	6.97	0.01
Sex+α+TP+(Sex*α)+(Sex*TP)	0.03	4	-6.68	22.14	7.67	0.01
Sex+α+(Sex*α)	0.00	3	-8.21	22.88	8.41	0.01
Sex+TP+(Sex*TP)	0.01	5	-6.63	24.46	9.99	0.00

[a] Sex = sex, Pop = population, α = percent reliance on benthic prey, and TP = trophic position; [b] The number of estimated parameters in the model including the intercept and variance; [c] Log-likelihood of the regression model; [d] Sample size corrected Akaike's Information Criterion; [e] The difference between the current model AIC_C and the AIC_C of the most parsimonious model; [f] Akaike's weight; the likelihood of the current model relative to others in the candidate set.

Table 3.S5: Ranking criteria and the structure of candidate models describing non-baseline-normalized total mercury concentrations in South Rolly Lake stickleback. All models are based on a sample size of 35 fish and include additive (+) and interaction (*) terms.

Model Structure[a]	R^2	k[b]	LogL[c]	AIC_C[d]	ΔAIC_C	w_i[f]
Sex+TP+(Sex*TP)	0.81	5	10.46	-8.85	0.00	0.68
Sex+α+TP+(Sex*TP)	0.80	6	10.61	-6.22	2.63	0.18
TP	0.76	3	5.43	-4.08	4.77	0.06
Sex+α+TP+(Sex*α)+(Sex*TP)	0.80	7	10.67	-3.19	5.66	0.04
Sex+TP	0.75	4	5.44	-1.54	7.31	0.02
α+TP	0.75	4	5.44	-1.54	7.31	0.02
Sex+α+TP	0.74	5	5.45	1.17	10.02	0.00
Sex+α+TP+(Sex*α)	0.74	6	5.59	3.82	12.67	0.00
Sex	0.30	3	-13.26	33.29	42.14	0.00
Sex+α	0.28	4	-13.18	35.69	44.54	0.00
Sex+α+(Sex*α)	0.27	5	-12.92	37.92	46.77	0.00
Null	0.00	2	-20.03	44.44	53.29	0.00
α	-0.03	3	-20.00	46.77	55.62	0.00

[a] Sex = sex, Pop = population, α = percent reliance on benthic prey, and TP = trophic position; [b] The number of estimated parameters in the model including the intercept and variance; [c] Log-likelihood of the regression model; [d] Sample size corrected Akaike's Information Criterion; [e] The difference between the current model AIC_C and the AIC_C of the most parsimonious model; [f] Akaike's weight; the likelihood of the current model relative to others in the candidate set.

Table 3.S6: Ranking criteria and the structure of candidate models describing non-baseline-normalized total mercury concentrations in Stormy Lake stickleback. All models are based on a sample size of 51 fish and include additive (+) and interaction (*) terms.

Model Structure[a]	R^2	k[b]	LogL[c]	AIC_C[d]	ΔAIC_C	w_i[f]
TP	0.14	3	9.50	-12.49	0.00	0.46
Sex+TP	0.13	4	9.67	-10.46	2.02	0.17
α+TP	0.13	4	9.61	-10.36	2.13	0.16
Sex+TP+(Sex*TP)	0.12	5	9.89	-8.44	4.04	0.06
Sex+α+TP	0.12	5	9.83	-8.32	4.16	0.06
Sex+α+TP+(Sex*α)	0.11	6	10.27	-6.64	5.85	0.02
Sex+α+TP+(Sex*TP)	0.11	6	10.04	-6.17	6.31	0.02
Null	0.00	2	5.08	-5.91	6.57	0.02
Sex	0.01	3	5.73	-4.95	7.54	0.01
Sex+α+TP+(Sex*α)+(Sex*TP)	0.10	7	10.34	-4.07	8.42	0.01
α	-0.02	3	5.16	-3.81	8.67	0.01
Sex+α	-0.01	4	5.79	-2.72	9.77	0.00
Sex+α+(Sex*α)	0.00	5	6.68	-2.04	10.45	0.00

[a] Sex = sex, Pop = population, α = percent reliance on benthic prey, and TP = trophic position; [b] The number of estimated parameters in the model including the intercept and variance; [c] Log-likelihood of the regression model; [d] Sample size corrected Akaike's Information Criterion; [e] The difference between the current model AIC_C and the AIC_C of the most parsimonious model; [f] Akaike's weight; the likelihood of the current model relative to others in the candidate set.

Table 3.S7: Ranking criteria and the structure of candidate models describing non-baseline-normalized total mercury concentrations in Tern Lake stickleback. All models are based on a sample size of 51 fish and include additive (+) and interaction (*) terms.

Model Structure[a]	R^2	k[b]	LogL[c]	AIC_C[d]	ΔAIC_C	w_i[f]
Null	0.00	2	-17.48	39.21	0.00	0.33
TP	0.00	3	-16.98	40.45	1.24	0.17
Sex	-0.01	3	-17.17	40.82	1.62	0.15
α	-0.02	3	-17.48	41.44	2.23	0.11
Sex+TP	-0.01	4	-16.73	42.30	3.09	0.07
α+TP	-0.02	4	-16.93	42.69	3.48	0.06
Sex+α	-0.03	4	-17.12	43.06	3.86	0.05
Sex+α+TP	-0.03	5	-16.62	44.51	5.30	0.02
Sex+TP+(Sex*TP)	-0.03	5	-16.73	44.73	5.53	0.02
Sex+α+(Sex*α)	-0.04	5	-16.96	45.19	5.98	0.02
Sex+α+TP+(Sex*α)	-0.04	6	-16.52	46.86	7.65	0.01
Sex+α+TP+(Sex*TP)	-0.05	6	-16.61	47.04	7.84	0.01
Sex+α+TP+(Sex*α)+(Sex*TP)	-0.07	7	-16.51	49.52	10.31	0.00

[a] Sex = sex, Pop = population, α = percent reliance on benthic prey, and TP = trophic position; [b] The number of estimated parameters in the model including the intercept and variance; [c] Log-likelihood of the regression model; [d] Sample size corrected Akaike's Information Criterion; [e] The difference between the current model AIC_C and the AIC_C of the most parsimonious model; [f] Akaike's weight; the likelihood of the current model relative to others in the candidate set.

Table 3.S8: Ranking criteria and the structure of global candidate models describing baseline-normalized total mercury concentrations in stickleback from six study lakes in the Cook Inlet Basin, Alaska. All models are based on a sample size of 291 fish and include additive (+) and interaction (*) terms.

Model Structure[a]	R^2	k[b]	LogL[c]	AIC_C[d]	ΔAIC_C	w_i[f]
Sex+Pop	0.47	8	-269.14	554.81	0.00	0.27
Sex+Pop+α	0.47	9	-268.66	556.00	1.19	0.15
Sex+Pop+TP	0.47	9	-268.70	556.07	1.25	0.15
Sex+Pop+α+TP	0.47	10	-268.21	557.25	2.43	0.08
Sex+Pop+TP+(Pop*TP)	0.47	14	-264.11	557.81	3.00	0.06
Sex+Pop+α+TP+(Pop*TP)	0.48	15	-263.11	558.06	3.25	0.05
Sex+Pop+TP+(Sex*TP)	0.46	10	-268.70	558.22	3.40	0.05
Sex+Pop+α+(Sex*α)	0.46	11	-267.91	558.81	4.00	0.04
Sex+Pop+α+TP+(Sex*TP)	0.46	11	-268.21	559.41	4.60	0.03
Sex+Pop+TP+(Sex*TP)+(Pop*TP)	0.47	15	-263.90	559.64	4.82	0.02
Sex+Pop+α+TP+(Sex*TP)+(Pop*TP)	0.47	16	-262.91	559.91	5.09	0.02
Sex+Pop+α+TP+(Sex*α)	0.46	12	-267.68	560.54	5.73	0.02
Sex+Pop+α+TP+(Sex*α)+(Pop*TP)	0.47	17	-262.80	561.96	7.15	0.01
Pop+TP+(Pop*TP)	0.46	13	-267.29	561.96	7.15	0.01
Sex+Pop+α+(Pop*α)	0.47	15	-265.18	562.19	7.38	0.01
Pop+α+TP+(Pop*TP)	0.46	14	-266.52	562.65	7.83	0.01
Sex+Pop+α+TP+(Sex*α)+(Sex*TP)	0.46	13	-267.68	562.75	7.93	0.01
Sex+Pop+α+(Sex*α)+(Pop*α)	0.47	16	-264.37	562.83	8.01	0.01
Sex+Pop+α+TP+(Pop*α)	0.47	16	-264.83	563.75	8.93	0.00
Sex+Pop+α+TP+(Sex*α)+(Sex*TP)+(Pop*TP)	0.47	18	-262.56	563.76	8.95	0.00
Pop	0.45	7	-274.80	564.02	9.21	0.00
Sex+Pop+α+TP+(Pop*α)+(Pop*TP)	0.48	21	-259.37	564.37	9.55	0.00
Sex+Pop+α+TP+(Sex*α)+(Pop*α)	0.47	17	-264.26	564.88	10.07	0.00
Pop+TP	0.44	8	-274.49	565.51	10.70	0.00
Pop+α	0.44	8	-274.60	565.74	10.92	0.00
Sex+Pop+α+TP+(Sex*TP)+(Pop*α)+(Pop*TP)	0.48	22	-258.91	565.81	11.00	0.00
Sex+Pop+α+TP+(Sex*TP)+(Pop*α)	0.46	17	-264.81	565.98	11.16	0.00
Sex+Pop+α+TP+(Sex*α)+(Pop*α)+(Pop*TP)	0.48	22	-259.01	565.99	11.18	0.00
Sex+Pop+α+TP+(Sex*α)+(Sex*TP)+(Pop*α)	0.46	18	-264.24	567.13	12.31	0.00
Pop+α+TP	0.44	9	-274.28	567.23	12.42	0.00
Sex+Pop+α+TP+(Sex*α)+(Sex*TP)+(Pop*α)+(Pop*TP)	0.48	23	-258.55	567.47	12.66	0.00
Pop+α+TP+(Pop*α)+(Pop*TP)	0.46	20	-263.16	569.61	14.80	0.00
Pop+α+(Pop*α)	0.44	14	-271.88	573.37	18.55	0.00
Pop+α+TP+(Pop*α)	0.44	15	-271.64	575.11	20.29	0.00
Sex	0.02	3	-355.68	717.44	162.63	0.00
Sex+α	0.02	4	-355.07	718.28	163.47	0.00
Sex+TP	0.02	4	-355.68	719.50	164.69	0.00

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Model Structure[a]	R^2	k[b]	LogL[c]	AIC_C[d]	ΔAIC_C	w_i[f]
Sex+α+(Sex*α)	0.02	6	-353.77	719.85	165.03	0.00
Sex+TP+(Sex*TP)	0.02	5	-354.89	720.00	165.19	0.00
Sex+α+TP+(Sex*TP)	0.02	6	-353.93	720.17	165.35	0.00
Sex+α+TP	0.02	5	-355.06	720.35	165.53	0.00
Sex+α+TP+(Sex*α)	0.02	7	-353.76	721.93	167.12	0.00
α	0.00	3	-357.97	722.03	167.21	0.00
Null	0.00	2	-359.06	722.16	167.34	0.00
Sex+α+TP+(Sex*α)+(Sex*TP)	0.02	8	-352.91	722.36	167.54	0.00
α+TP	0.00	4	-357.95	724.05	169.23	0.00
TP	0.00	3	-359.05	724.20	169.38	0.00

[a] Sex = sex, Pop = population, α = percent reliance on benthic prey, and TP = trophic position; [b] The number of estimated parameters in the model including the intercept and variance; [c] Log-likelihood of the regression model; [d] Sample size corrected Akaike's Information Criterion; [e] The difference between the current model AIC_C and the AIC_C of the most parsimonious model; [f] Akaike's weight; the likelihood of the current model relative to others in the candidate set.

Chapter 4: Breeding season mercury dynamics in threespine stickleback: differences between sexes and ecotypes[1]

4.1 Abstract

Temporal trends in fish mercury concentrations are widely recognized as an important aspect of variation in Hg exposure to people and wildlife. I measured total mercury concentrations in threespine stickleback (*Gasterosteus aculeatus*) from Benka Lake, Alaska, USA over the course of the summer breeding season in order to 1) identify temporal trends in total mercury concentration of fish tissues, 2) determine whether these trends differed between females and males and between benthic and limnetic ecotypes, and 3) examine the roles of several biological factors in determining these trends. I found that stickleback total mercury concentrations were related by a quadratic function to date in males of both ecotypes and to a lesser degree in benthic females, but not in limnetic females. When the effects of trophic position, habitat-specific foraging, body condition, and size were examined independently for each of the sexes and ecotypes, I found that trophic position had the strongest effect on THg concentrations in all sex and ecotype combinations, but the magnitude of this effect was greater in the benthic ecotype. The importance and effect size of the remaining parameters varied substantially between the

[1] Willacker, J. J., C. A. Eagles-Smith, F. A. von Hippel, and T. M. O'Hara. Temporal mercury dynamics in threespine stickleback: differences between sexes and ecotypes. Prepared for submission to *Environmental Science & Technology*.

sexes and ecotypes, though the direction of the relationships was consistent across groups. These results demonstrate the importance of examining intra-population variation in the factors underlying mercury bioaccumulation and suggest that changes in methylmercury production are not solely responsible for fluctuations in fish mercury concentrations during the summer growing season.

4.2 Keywords

benthic, body condition, *Gasterosteus aculeatus*, habitat-specific foraging, limnetic, stable isotopes, trophic position

4.3 Introduction

Mercury (Hg) is perhaps the most widely distributed and pervasive environmental contaminant in aquatic systems around the globe.[1] Methylmercuries (MeHg+), the organic and more bioavailable species of Hg, are of particular concern as they are the most abundant form in most biotic matrices,[2] are known to biomagnify,[3] and are the most toxicologically potent form of Hg.[4] Mercury is primarily emitted as inorganic species[5] which are minimally available for uptake into aquatic food webs, but in many aquatic habitats can be rapidly biotransformed into MeHg+.[1] The rate and efficiency of this conversion is known to vary with many physical, chemical, and biological characteristics of systems, resulting in substantial variation in MeHg+ levels of water bodies even when they are adjacent to one another and have presumably similar Hg sources.[6-8]

The uptake and flow of Hg through food webs is further complicated by the effects and interactions of numerous community, population, and individual characteristics that

alter the accumulation of Hg in biota and thus confound attempts to understand patterns of accumulation across systems. Thus, Hg concentrations in biota do not always reflect the magnitude of Hg loadings to the system, nor necessarily the availability of MeHg+ in abiotic matrices, but rather integrate these components with the complex ecological effects occurring at numerous hierarchical levels.

Understanding the factors and interactions that govern patterns of Hg accumulation and biomagnification is important to our ability to identify systems or species to which Hg poses a risk. However, these factors confound one another and are often specific to a particular sex, species, location, time point, or system. Despite these complications, some generalizable relationships exist. For example, across many ecosystem types and species there is a consistent relationship between Hg concentrations and the trophic position (TP) of consumers.[3, 8, 9] Similarly, Hg concentrations typically increase with fish size[10] and age[11] due to increased lifelong bioaccumulation and in some cases also due to diet shifts to more contaminated (e.g., higher TP) prey.[12] Growth dynamics also influence Hg concentrations, with higher growth rates associated with the dilution of Hg and thus lower tissue Hg concentrations.[13, 14] However, the physiological complexities of growth and difficulty of measuring growth in the field make it challenging to apply these concepts in many wild populations. Similarly, it is difficult to predict the effects of habitat-specific foraging (e.g., use of littoral versus limnetic resources) on Hg concentrations because both the magnitude and direction of the relationship varies with the ecological context of the system[12, 15-21] and the scale at which the comparison is made (chapter 3).

In many species Hg concentrations differ between females and males, with females typically, though not always (chapter 3),[12, 22] having lower Hg concentrations than males.[19, 23-31] This difference has been attributed to losses of Hg in eggs,[24, 31] though there is little evidence for this.[23, 28] It has also be ascribed to differences in the foraging ecologies of the sexes[19, 30] and differences in the growth dynamics of females and males.[22, 26, 27, 29, 30]

Further, an increasing body of literature has demonstrated that Hg concentrations in fish are often temporally variable.[32-37] The degree to which the cause of this variability is due to changes in the production and concentration of bioavailable Hg over the summer season[33, 36, 37] versus changes in the underlying physiological determinants of Hg concentrations discussed above is unclear.[32, 35, 38]

To better understand the basis for sex specific differences and seasonal variability in the Hg concentration of fishes, I measured total Hg (THg) concentrations in female and male threespine stickleback fish (*Gasterosteus aculeatus*) from Benka Lake, Alaska, USA each week over the course of a 12 week summer breeding season. The stickleback in Benka Lake have been widely studied because it is the only Alaskan lake in which both benthic (i.e., foraging primarily in the littoral zone of the lake on benthic macroinvertebrates) and limnetic (i.e., primarily foraging on zooplankton in the open water, limnetic zone of the lake) ecotypes of stickleback have been found.[39-41] Within Benka Lake these ecotypes have distinct morphologies, diets, and life histories similar to those seen in allopatric populations of stickleback, though the Benka Lake ecotypes have less distinct diets and morphologies than observed in the allopatric ecotypes.[19, 39-41]

Previous work at Benka Lake by Willacker et al.[19] indicated that these ecological differences result in higher THg concentrations in the limnetic ecotype compared to the benthic ecotype. Further, Willacker et al.[19] demonstrated that female stickleback in Benka Lake have lower THg concentrations than males of their ecotype. While these differences were attributed in part to differing TPs and foraging ecologies of the sexes and ecotypes, detailed examination of the mechanisms underlying these differences were beyond the scope of that work. The current study couples data on short-term temporal variability in THg concentrations with data on the TP, habitat use, relative condition, and size of individual fish in order to identify the relative importance of each factor in determining THg bioaccumulation over the course of the breeding season. Together these data allow for the temporal variability due to an individual's ecological and physical characteristics to be accounted for and thus provide insights into the processes underlying Hg dynamics.

4.4 Materials and Methods

4.4.1 Study site

Benka Lake (62.1875° N, 150.0040° W) is a small (< 0.5 km^2) lake located approximately 125 km north of Anchorage in the Cook Inlet Basin of Alaska. The lake lacks inlets or outlets, occupies a small watershed (~2.5 km^2), and is relatively pristine with minimal shoreline development (e.g., shoreline clearing for residences) and no known local sources of Hg. Thus, atmospheric deposition is most likely the primary source of Hg to Benka Lake.

4.4.2 Sample collection

Stickleback were sampled every Monday between May 22 and August 6, 2012 from two sites within Benka Lake. One site is a known breeding area for the benthic ecotype while the other site is a known breeding area for the limnetic ecotype.[39-41] Fish were trapped using unbaited 0.6 cm wire mesh minnow traps set from shore, euthanized with an overdose of buffered MS-222 anesthetic, rinsed with lake water, and stored on ice while in the field (< 4 hours), then stored at -80°C in the laboratory. In order to account for differences in the isotopic baselines of benthic and limnetic food webs, gastropods (*Helisoma anceps*) and mussels (*Anodonta beringiana*) were collected at the beginning and end of the field season from the same areas as stickleback and preserved in the same manner.

4.4.3 Sample preparation

For each sampling date I analyzed approximately 15 fish of each sex and ecotype for a total of 667 fish. Only reproductively mature fish (based on gonad dissections and secondary sexual characteristics) were utilized in order to ensure accurate sex assignments. Since most stickleback in Benka Lake spawn at 2 years of age,[39] utilizing only reproductive individuals also minimized variation in Hg concentrations associated with differences in age. I assigned each fish a unique identifier, weighed the lightly blotted fish to the nearest 0.1 mg, and measured standard length (SL; anterior tip of premaxilla to posterior border of hyperal plate) to the nearest 0.1 mm. I then removed the gastrointestinal contents and dissected out the eggs (in females), as well as the kidney and liver (both sexes), prior to returning the empty gastrointestinal tract to the body cavity.

Egg, liver, and kidney tissue was retained for a related study on Hg biodynamics in stickleback tissues. The macro-parasites *Schistocephalus solidus* and *Hysterothylacium* sp. were also removed prior to analyses.

Eviscerated stickleback carcasses were reweighed to the nearest 0.1 mg then dried at 50˚C until a constant weight was achieved (~48 hr.). Following drying, carcasses were weighed again to the nearest 0.1 mg before being ground into a fine powder using a cryogenic tissue mill (SPEX SamplePrep, Metuchen, NJ, USA). Gastropod and mussel samples were removed from their shells and processed similarly to fish with the exception that mussels were dried for approximately 72 hr.

4.4.4 Stable isotope analysis

I determined stable carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) isotope ratios at the University of Alaska Anchorage – Environmental and Natural Resources Institute Stable Isotope Laboratory using a Thermo-Finnigan Delta Plus XP Isotope Ratio Mass Spectrometer (IRMS) coupled to a Costech 4010 Elemental Combustion System. Isotope ratios are presented in standard δ notation as parts per thousand (‰) differences between the isotope ratio of the sample and that of a standard (Vienna Pee Dee Belemnite for carbon and air for nitrogen). Additionally, the molar ratio of carbon to nitrogen (C:N) was calculated and utilized as a proximate condition index since C:N is positively correlated with lipid content[42] and thus overall fish condition.[43-45] Quality assurance – quality control included six replicates each of a stable isotope reference material (SRM 1547: peach leaves, available from the National Institute of Standards and Technology; $\delta^{13}C = -25.89 \pm 0.3‰$, $\delta^{15}N = 1.90 \pm 0.3‰$) and laboratory working standard (Cheney

Lake stickleback; $\delta^{13}C = -29.48 \pm 0.2‰$, $\delta^{15}N = 9.77 \pm 0.1‰$) with every batch of 40 samples. External instrument reproducibility for carbon isotope analysis was ± 0.2‰ and ± 0.1‰ for nitrogen analysis. Isotope values were not lipid normalized because lipid content is relatively low in stickleback carcasses (C:N = 3.77 ± 0.02, n = 667).

4.4.5 Mercury Analysis

Mercury concentrations were determined at the U.S. Geological Survey, Forest and Rangelands Ecosystem Science Center in Corvallis, OR using a DMA-80 Direct Mercury Analyzer (Milestone Inc., Shelton, CT, USA) and following EPA method 7473.[46] Quality assurance measures included analysis of two certified reference materials (CRM; dogfish muscle tissue [DORM-4], 0.41 μg/g dw and lobster hepatopancreas [TORT-2], 0.27 μg/g dw; National Research Council of Canada, Ottawa, Canada), system blanks, method blanks, calibration standards and two duplicates per batch of 40 samples. Recoveries averaged 99.3 ± 0.5% (*n* = 44) for CRMs and 97.8 ± 0.9% (*n* = 66) for calibration standards. Relative percent difference (RPD) for all duplicates averaged 1.1 ± 0.1% (n = 43). Mercury concentrations are presented as ng/g dry weight, but can be converted to wet weight estimates using the mean percent moisture of stickleback used in this study (77.6% ± 0.09%, n = 667).

4.4.6 Statistical analysis

Prior to analysis, THg concentrations were natural-log transformed in order to meet the assumptions for parametric modeling. Stable isotope data were used to calculate reliance on benthic prey (α) and trophic position (TP) as described in Willacker et al.[19] All analyses were conducted in R version 2.15.0[47] and, unless otherwise noted, data are

presented as back-transformed least-square means and standard errors from the model outputs.

I evaluated the effects of sex, ecotype, sampling date, TP, α, C:N, and SL as well as interactions between sex, ecotype, and each of the covariates using a linear mixed-effects model. In order to account for potential non-linear relationships between stickleback THg concentration and date I also included a quadratic date term. These analyses were structured in a repeated measures framework by including the random effects of sex, ecotype, and date as a composite random variable. This global model included significant interactions between both factors and the covariates, thus subsequent analyses were conducted separately for each sex and ecotype. I assessed whether inclusion of the quadratic date variable improved model fit over linear only models for each sex-ecotype separately using likelihood ratio tests. Multiple regression coupled with quantitative model selection techniques were then used to examine the effects of TP, α, C:N, and SL on stickleback THg concentrations. Sample size corrected Akaike's Information Criterion (AIC_C) was used to select the most parsimonious model for explaining THg concentrations from an initial set of 64 *a priori* candidate models (Tables 4.S1 – 4.S4) for each sex-ecotype. I ranked the candidate models using the AIC_C differences between the best model and the other candidate models (ΔAIC_C).[48] The Akaike weights (w_i) of candidate models were compared to assess each model's probability of being the best fitting model.

To evaluate the relative importance of each covariate across models, I calculated variable weights (V).[48] I also conducted full-set model averaging and utilized model

averaged beta-coefficients to assess the "true" effect size of each variable on THg concentrations.[48] Model averaging does not rely on the selection of a single "best" model and thus incorporates model selection uncertainty. Therefore, model averaging provides more robust coefficient estimates than traditional regression techniques.[48]

Finally, model averaged estimates were used to predict THg concentrations in each sex-ecotype at five points over the observed range of each covariate when the other covariates were held constant at their mean across groups. This technique is analogous to the calculation of partial residuals.[48] Analysis of covariance (ANCOVA) was then utilized to assess differences in the relationship between THg concentration and each covariate. I employed the Bonferroni correction to maintain a family-wise error rate of 0.05 among the ANCOVAs.

4.5 Results

All parameters differed significantly between ecotypes and sexes (two factor ANOVAs $p > 0.05$) with the exception that there was no significant difference in the C:N of benthic and limnetic fish (Table 4.1).

The mean THg concentration across all fish sampled was 136 ± 2 ng g^{-1} dw but there was substantial variation among individuals with THg concentrations ranging from 30 to 328 ng g^{-1} dw. Mercury concentrations were lower in the benthic than in the limnetic ecotype ($F_{1,665} = 44.35$, $p < 0.001$) and in females compared to males ($F_{1,665} = 449.88$, $p < 0.001$; Table 4.1). Over the course of the 12 week study period THg concentrations in benthic and limnetic males increased by approximately 36 and 46%,

respectively then decreased (71% in benthic males, 63% in limnetic males). This decline resulted in a net decrease of 60% for benthic males and 46% for limnetic males in THg concentration over the course of the breeding season. The THg concentrations in females of both ecotypes also increased initially and then declined; however, the changes were less than in males (Figure 4.1).

My initial global model indicated that there were significant sex × date ($F_{1,39} = 4.66$, $p = 0.037$), sex × C:N ($F_{1,607} = 6.21$, $p = 0.013$), ecotype × α ($F_{1,607} = 4.37$, $p = 0.037$), and ecotype × TP ($F_{1,607} = 7.03$, $p = 0.008$) interactions. Therefore, I subsequently examined each sex-ecotype separately. The likelihood ratio tests indicated that the inclusion of the quadratic date term significantly improved model fit in benthic females ($\chi^2 = 6.47$, n = 175, $p = 0.012$), benthic males ($\chi^2 = 8.79$, n = 168, $p = 0.003$), and limnetic males ($\chi^2 = 9.85$, n = 163, $p = 0.002$), but not in limnetic females ($\chi^2 = 3.39$, n = 161, $p = 0.065$). Therefore, the quadratic date terms were retained in subsequent models.

Based on quantitative model selection the model containing all covariates was the most parsimonious for benthic females ($w_i = 0.47$; Table 4.S1), benthic males ($w_i = 0.35$; Table 4.S2), and limnetic males ($w_i = 0.67$; Table 4.S3). This model was the only plausible ($\Delta AIC_c < 2$) model for benthic females and limnetic males, whereas two other models, one without SL ($w_i = 0.22$) and the other without C:N ($w_i = 0.17$), were also plausible for benthic males. The most parsimonious model for limnetic females included C:N, SL, and TP ($w_i = 0.27$; Table 4.S4), but was only 1.2 times more likely than the next best model which included the date terms ($w_i = 0.23$).

Across ecotype and sex, variable weights indicated that TP was consistently the most important variable measured (V = 1.00; Table 4.2); however, there was substantial variability in the importance of the remaining variables within each sex-ecotype. For benthic females, SL was as important as TP (V = 1.00) and was followed in importance by C:N (V = 0.87), date (V = 0.84), and α (V = 0.75). For benthic males, date (V = 0.94) was the second most important variable and importance progressively declined from α (V = 0.92) to C:N (V = 0.72) to SL (V = 0.64). In addition to TP, C:N and SL both had a relative importance of 1.00 for limnetic females. In contrast, date and α were relatively unimportant (V = 0.47 and 0.25, respectively) for limnetic females. For limnetic males, SL and date were nearly as important as TP (V = 0.99 and 0.97, respectively) while C:N (V = 0.88) and α (V = 0.84) were slightly less important. Similar conclusions were drawn from model averaged beta-coefficients; variables with low variable weights in a given model set had 95% confidence intervals of the coefficient estimates that overlapped zero (Table 4.3).

Using model averaged estimates I examined the effects of individual variables when all other variables were held constant. Accounting for the effects of all variables except sex, I found that mean THg concentrations were higher in limnetic males (165 ± 12 ng g^{-1} dw) than limnetic females (121 ± 5 ng g^{-1} dw), but the difference between benthic males (145 ± 11 ng g^{-1} dw) and benthic females (138 ± 8 ng g^{-1} dw) was not significant (Figure 4.2). Model averaged estimates also showed that of the five variables examined, TP had the largest effect on THg concentrations in all sex-ecotypes, with THg concentrations increasing by approximately 575% in benthic fish and 175 and 250% in limnetic males

and females, respectively (Figure 4.3a). Importantly, ANCOVA indicated that while intercepts were the same between ecotypes and only marginally different between sexes ($F_{1,14} = 5.01$, $p = 0.041$), the slope of the relationship between TP and THg was the same regardless of sex but significantly different between the benthic and limnetic ecotypes ($F_{1,14} = 10.40$, $p = 0.006$).

The magnitudes of the effects of the remaining variables differed among sex-ecotypes, though the directions of the effects were consistent across groups. Total Hg concentrations were negatively correlated with C:N ($F_{1,14} = 29.08$, $p < 0.001$) and the effect of C:N ranged from 23% in benthic males to 67% in limnetic females, with the limnetic ecotype displaying significantly greater effects ($F_{1,14} = 5.49$, $p = 0.034$) than the benthic ecotype (Table 4.4; Figure 4.3b). Standard length was positively correlated with THg concentrations ($F_{1,14} = 68.98$, $p < 0.001$) in both sexes and ecotypes; however, males had a higher intercept than females ($F_{1,14} = 25.91$, $p < 0.001$) and the benthic ecotype had a marginally lower slope than the limnetic ecotype ($F_{1,14} = 4.60$, $p < 0.050$; Figure 4.3c). When the effects of TP, SL, and C:N are accounted for, α and THg concentrations displayed a positive relationship ($F_{1,14} = 45.57$, $p < 0.001$) which varied by sex ($F_{1,14} = 17.08$, $p = 0.001$) but not ecotype ($F_{1,14} = 2.22$, $p = 0.158$; Figure 4.3d).

When all other covariates were accounted for, there remained a substantial effect of date on THg concentrations (Figure 4.4). Female stickleback of both ecotypes displayed a net increase in THg concentration between the beginning and end of the breeding season, though the increase was more substantial for benthic females (25%) than for limnetic females (4%). In contrast, males of both ecotypes experienced a net loss of THg over the

breeding season, ending with THg concentrations 11% (benthic males) and 23% (limnetic males) lower than their starting concentrations. However, the relationship between date and THg concentration was not linear in three of the four sex-ecotypes and thus these figures do not adequately capture the temporal variation (Figure 4.4).

As with the raw data, the model averaged temporal changes of males were more pronounced than those of females. After accounting for the effects of all covariates, benthic and limnetic males followed a consistent trajectory with their THg concentrations increasing by 49 and 30%, respectively, between the start of sampling on May 22 and their peak on June 25. Their THg concentrations then declined by 40 and 41% toward the end of the sampling period in early August. The temporal trends of females differed between the benthic and limnetic ecotypes, with benthic females displaying a quadratic trend in which THg concentrations initially increased by 38% and then declined by 10%, while the relationship between THg and date for limnetic females was approximately linear.

4.6 Discussion

I found substantial temporal variation in THg concentrations of threespine stickleback fish from Benka Lake, with mean THg concentrations fluctuating by as much as 70% over the course of the three month breeding season (Figure 4.1). Importantly, these temporal trends were significantly different for males and females, but not markedly different between ecotypes. Specifically, male stickleback displayed a more distinctly quadratic relationship between their THg concentrations and date whereas in

females this curve was less pronounced. Many differences also existed in the relationships between THg concentrations and individual covariates for both sexes and ecotypes, suggesting that even when Hg concentrations are similar, the underlying processes regulating bioaccumulation may differ.

Male stickleback of both ecotypes displayed a strongly quadratic relationship between date and THg concentration, with Hg concentrations initially increasing to a peak in late June and then declining towards the end of the sampling period (Figure 4.1). Similar trends have been observed in stickleback and mudsucker (*Gillichthys mirabilis*) from multiple wetlands in San Francisco Bay, CA, USA[37] and in mimic shiner (*Notropis volucellus*) from Wisconsin, USA.[49] Changes in MeHg+ production and subsequent accumulation have repeatedly been identified as a likely cause of this pattern.[33, 36, 37] Methylmercury production typically increases during the summer active season[50-52] which, coupled with the potentially rapid uptake of MeHg+ by small fish[53], could explain temporal patterns of THg concentration in fishes.

However, as noted by Eagles-Smith and Ackerman,[37] this hypothesis cannot explain why fish Hg concentrations start declining during the peak of the active season, when MeHg+ production should continue to be high. Further, if the observed temporal pattern depended solely on changes in the production of MeHg+, then I would expect that individuals most closely coupled to benthic habitats, where the majority of MeHg+ production occurs, would have the greatest response. This is not observed in Benka Lake stickleback where the quadratic pattern is evident in both benthic and limnetic males, but to a lesser degree in benthic females which had a higher mean reliance on benthic

resources than any other group. These data suggest that other factors are important in determining temporal patterns in THg concentrations.

Temporal changes in foraging habits, size, or body condition can also contribute to concurrent changes in Hg concentrations.[32, 37] I tested this by examining the individual effects of TP, body condition, α, and body size on temporal trends in Hg concentration.

In both sexes and ecotypes TP was consistently among the most important determinants of THg concentrations in Benka Lake stickleback. This result is in agreement with previous work that has attempted to parse variance between TP and other ecological variables (chapter 3).[12, 19, 54] While the importance of TP in determining THg concentrations has been widely recognized, our current knowledge of Hg biomagnification suggests that biomagnification rates are determined by bioenergetic assimilation efficiencies[55] and are thus fairly uniform among species, food webs, ecosystem types, and geographical regions.[3, 56, 57] The current study suggests a more complicated relationship.

When all other covariates were held constant, I found that TP had a larger effect size in benthic than in limnetic stickleback (Figure 4.3a). This result could be due to higher Hg availability in benthic habitats than in limnetic habitats;[3] however, in Benka Lake this is unlikely because benthic primary consumers have lower THg and MeHg+ concentrations than their limnetic counterparts.[19] This result is also unlikely to arise from differences in growth rates since adults (age 2) of the benthic ecotype attain a larger mean size than those of the limnetic ecotype which are the same age (Table 4.1). Elevated growth rates should result in growth dilution of Hg in the benthic ecotype.[13, 58] Similarly,

since the elimination of MeHg+ is slowed at lower temperatures,[59] and limnetic habitats in Benka Lake are consistently cooler than benthic habitats,[19] it is unlikely that differences in the elimination of Hg from stickleback tissues could result in the observed pattern.

If different assimilation rates of Hg between ecotypes exist, their cause is unclear; however, it may arise from differences in the nutritional composition of prey. For example, Gobas et al.[60] found that dichlorodiphenyltrichloroethane (DDT) was assimilated more efficiently from lipid rich prey than from lipid poor prey due to routing of the lipid component. Protein also preferentially assimilates when dietary concentrations are high.[61, 62] Since the majority of Hg in biota is bound to protein,[2] higher protein assimilation could be accompanied by higher Hg assimilation efficiencies. Thus, higher protein contents in benthic invertebrates compared to pelagic zooplankton[63] could explain increased biomagnification of Hg in the benthic compared to the limnetic ecotype. Indeed, C:N ratios, which are typically low in protein rich tissues,[61] are significantly lower in Benka Lake benthic invertebrates (4.0 ± 0.1) compared with zooplankton (7.4 ± 1.2).

I found that C:N, a proxy for the lipid content and thus the relative condition of fishes,[42] was negatively correlated with THg concentrations in all groups (Figure 4.3b). This pattern is consistent with the concept of growth dilution which suggests that the Hg concentrations of rapidly growing individuals, which are those with higher body conditions, are reduced compared to slower growing individuals.[13, 58] Similar negative

relationships between body condition and Hg concentration have been demonstrated for numerous fish species.[54, 64-66]

My data demonstrate that the effect of C:N on THg concentrations was much larger for the limnetic compared to the benthic ecotype of stickleback (Figure 4.3b). These results could arise from differences in the composition of prey in the two food webs (as discussed above) or differences in metabolic efficiencies of the two ecotypes. While the relationship between growth rate and Hg concentrations has been increasingly recognized,[13, 14, 26, 67] as far as I are aware only Scott[64] has previously reported inter-population variation in the slope of this relationship. Scott[64] examined the relationship between body condition and Hg concentration in multiple age classes of four fish species from two lakes and found that while most relationships were negative, there was significant variation in the magnitude of the slopes among species and in some cases age classes within a species. In contrast, Cizdziel et al.[66] found that the relationship between condition and Hg concentration was consistent for striped bass (*Morone saxatilis*) regardless of their location within Lake Mead, USA. While my data do not allow for the determination of why these relationships differ in benthic and limnetic stickleback of Benka Lake, they highlight the need to examine and account for such differences.

Previously, Willacker et al.[19] reported that THg concentrations were negatively correlated with α in stickleback. The current study demonstrates a similar negative relationship between α and THg concentrations; however, when the effects of TP, C:N, SL, and date are controlled for, this relationship is reversed (Figure 4.3d). Thus, the typical effects of habitat-specific foraging may reflect the correlated effects of multiple

ecological and metabolic parameters, such as TP, condition, and body size. The residual effect of α on THg concentration, after the contributions of other variables are removed, would likely reflect local differences in the bioavailability and concentration of Hg and thus would be expected to increase in benthic habitats. A similar conclusion was reached when the relationship between habitat-specific foraging and stickleback THg concentrations was examined across multiple populations (chapter 3).

After accounting for the effects of TP, C:N, SL, and α there remained significant variation in THg concentrations over the course of the breeding period, though the magnitude of this variation was dampened (Figure 4.4). This remaining temporal variation may more accurately reflect changes in the availability of MeHg+ during the summer season,[33, 36, 37] though this conclusion would have to be confirmed with *in situ* measurements of MeHg+ production as other explanations are also plausible. For example, the residual temporal pattern may result from other physiological factors such as internal redistribution of Hg into organ tissues[68] or temporal changes in the nutritional composition of prey items.

Overall my findings indicate that the temporal patterns of THg observed in small fishes are likely the result of numerous physiological and ecological processes and thus do not simply reflect increased production of bioavailable MeHg+ during the summer growing season. Further, I found that in Benka Lake stickleback the importance and magnitude of these factors in determining THg concentrations varied between sexes and ecotypes. My results underscore the importance of adequately characterizing the population(s) and cohorts being examined and accounting for multiple concurrent

changes in fish trophic states, condition, and size over the period of sampling. Future research should progress beyond accounting for these factors to the determination of the mechanistic processes underlying their effects and interactions.

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4.9 Tables

Table 4.1: Mean ± standard error for variables measured in female and male stickleback ecotypes sampled from Benka Lake, Alaska between May and August 2012. Geometric means are presented for total mercury and arithmetic for other variables.

Sex - ecotype	n	TP[a]	C:N[b]	SL[c] (mm)	α[d]	THg[e] (ng g^{-1} dw)
Benthic						
Female	175	3.98 ±0.04	3.66 ±0.04	55.5 ±0.4	0.52 ±0.01	104 ±0.1
Male	163	4.36 ±0.04	3.93 ±0.05	56.3 ±0.4	0.43 ±0.01	120 ±0.1
Limnetic						
Female	168	4.56 ±0.03	3.76 ±0.04	53.4 ±0.3	0.36 ±0.01	126 ±0.1
Male	161	4.68 ±0.03	3.74 ±0.04	55.4 ±0.4	0.36 ±0.01	155 ±0.1

[a] Trophic position; [b] carbon to nitrogen ratio; [c] standard length; [d] the proportion of the diet from benthic sources; [e] total mercury concentration.

Table 4.2: Relative importance of trophic position (TP), carbon to nitrogen ratio (C:N), standard length (SL), date, and reliance on benthic prey (α) in determining total mercury concentrations in stickleback sampled from Benka Lake, Alaska between May and August 2012.

Sex-ecotype	TP	C:N	SL	Date	α
Benthic					
Females	1.00	0.99	0.87	0.83	0.75
Males	1.00	0.64	0.72	0.94	0.92
Limnetic					
Females	1.00	1.00	1.00	0.47	0.25
Males	1.00	0.99	0.88	0.96	0.84

Table 4.3: Model averaged beta coefficients for the relationships between trophic position (TP), carbon to nitrogen ratio (C:N), standard length (SL), date, and reliance on benthic prey (α) with total mercury concentrations in stickleback sampled from Benka Lake, Alaska between May and August 2012.

Sex-ecotype	**TP**	**C:N**	**SL**	**Date**	**α**
Benthic					
Females	0.718 ± 0.15	-0.172 ± 0.16	0.014 ± 0.01	0.061 ± 0.06	0.459 ± 0.44
Males	0.709 ± 0.19	-0.128 ± 0.13	0.009 ± 0.01	0.120 ± 0.09	0.739 ± 0.54
Limnetic					
Females	0.468 ± 0.16	-0.388 ± 0.1	0.020 ± 0.011	0.021 ± 0.04	0.028 ± 0.56
Males	0.378 ± 0.21	-0.170 ± 0.1	0.014 ± 0.008	0.100 ± 0.07	0.680 ± 0.59

Table 4.4: Analysis of covariance results comparing the relationships between model averaged estimates of total mercury concentration and each of four covariates in stickleback sampled from Benka Lake, Alaska between May and August 2012. Degrees of freedom for all tests are 1 and 14.

Covariate[a]	Effect	F-value	*P*-value
TP			
	Sex	**5.09**	**0.041**
	Ecotype	0.09	0.773
	Sex × TP	0.17	0.690
	Ecotype × TP	**10.40**	**0.006**
C:N			
	Sex	**29.08**	**<0.001**
	Ecotype	1.89	0.191
	Sex × C:N	3.00	0.105
	Ecotype × C:N	**5.49**	**0.035**
SL			
	Sex	**25.91**	**<0.001**
	Ecotype	0.08	0.781
	Sex × SL	1.93	0.187
	Ecotype × SL	**4.60**	**0.050**
α			
	Sex	**26.14**	**<0.001**
	Ecotype	0.01	0.932
	Sex × α	**17.08**	**0.001**
	Ecotype × α	2.22	0.158

[a] TP - Trophic position; C:N - carbon to nitrogen ratio; SL - standard length; α - the proportion of the diet from benthic sources.

4.10 Figures

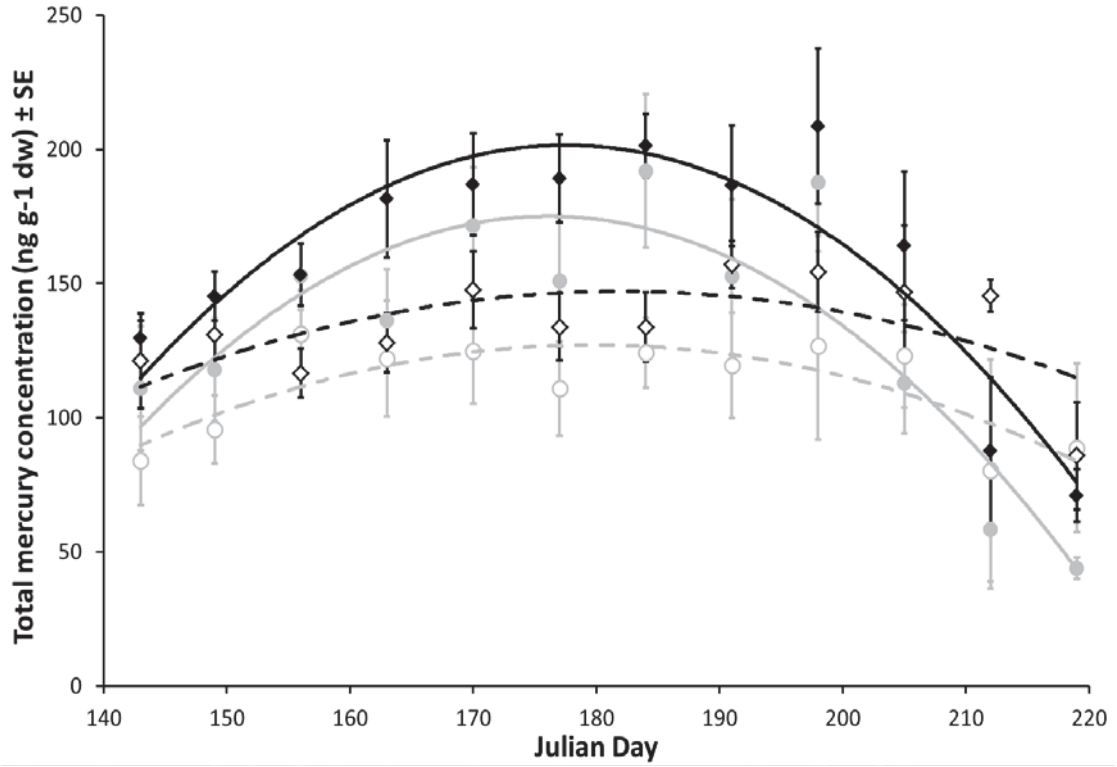

Figure 4.1: Temporal variation in total mercury concentrations of stickleback from Benka Lake, Alaska during the 2012 summer breeding season. Both females (open symbols, dashed lines) and males (solid symbols, solid lines) are displayed for benthic (grey) and limnetic (black) ecotypes.

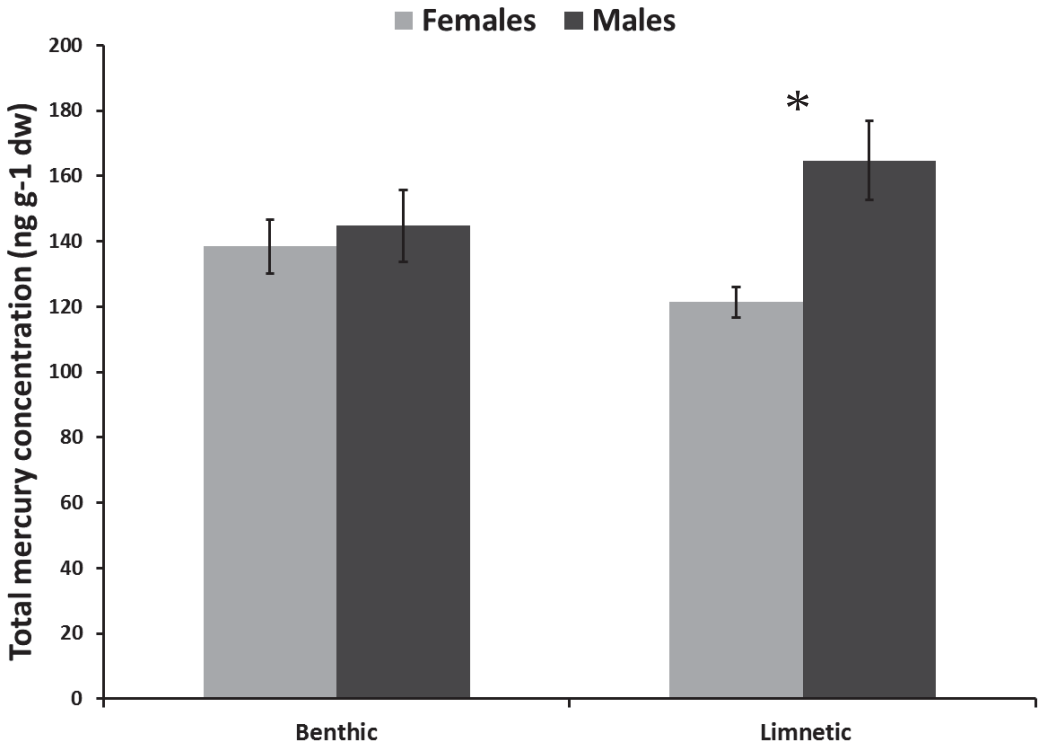

Figure 4.2: Back-transformed, model averaged estimates of mean (± unconditional SE) total mercury concentrations in female (grey) and male (black) benthic and limnetic ecotypes of stickleback from Benka Lake, Alaska after accounting for differences in trophic position, carbon to nitrogen ratio, standard length and proportion of benthic diet. Statistical significance between sexes is indicated by an asterisk (*) and was determined by the presence of non-overlapping standard error bars.

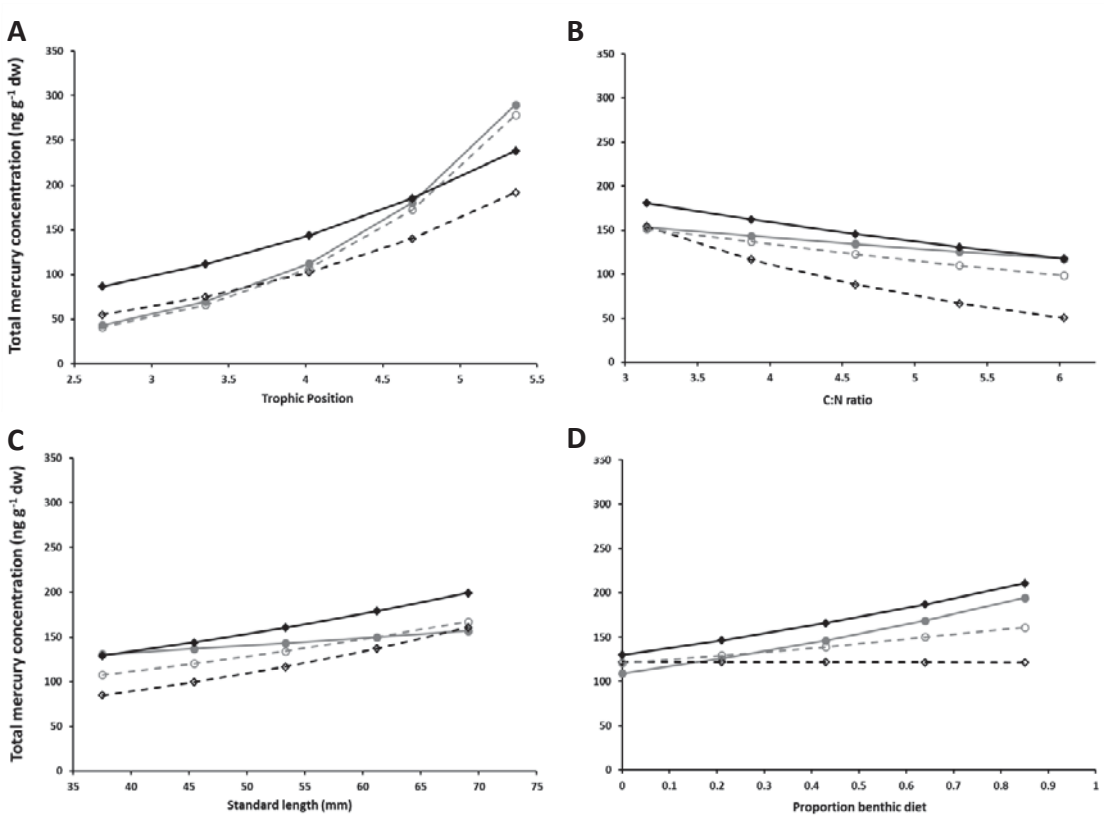

Figure 4.3: Model averaged estimates of changes in total mercury concentration over the observed range of trophic position (A), carbon to nitrogen (C:N) ratio (B), standard length (C), and proportion of benthic diet (D) in stickleback from Benka Lake, Alaska after accounting for the effects of all other measured variables including date. Both females (open symbols, dashed lines) and males (solid symbols, solid lines) are displayed for benthic (grey) and limnetic (black) ecotypes.

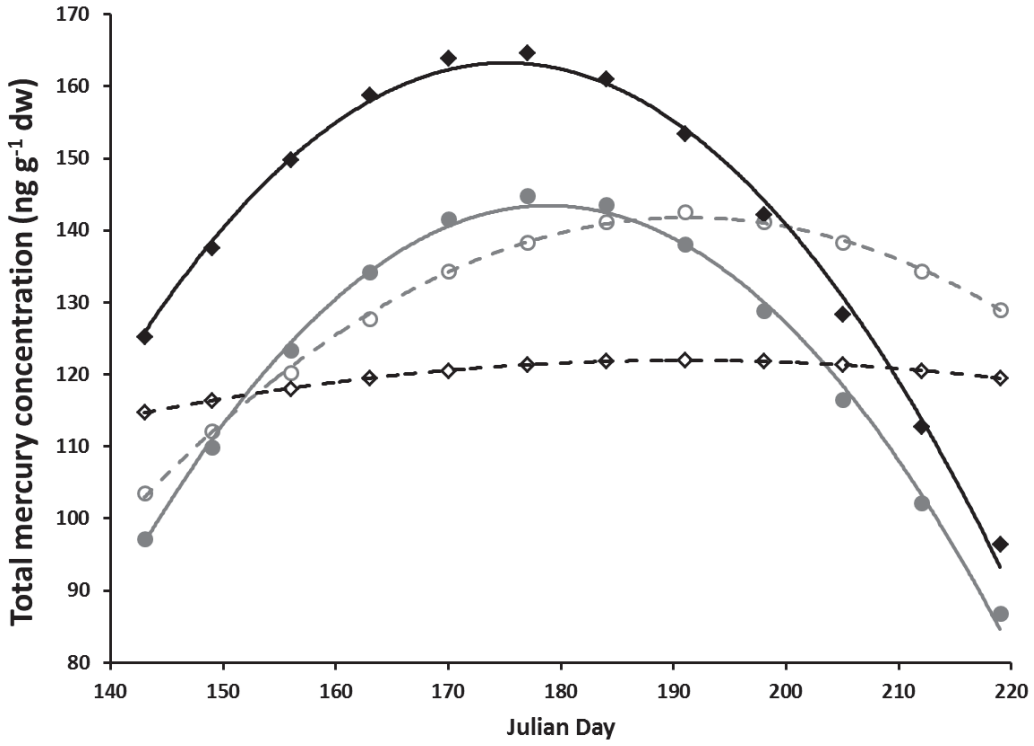

Figure 4.4: Model averaged estimates of temporal changes in total mercury concentrations of stickleback from Benka Lake, Alaska during the 2012 summer breeding season after accounting for differences in trophic position, carbon to nitrogen ratio, standard length, and proportion of benthic diet. Both females (open symbols, dashed lines) and males (solid symbols, solid lines) are displayed for benthic (grey) and limnetic (black) ecotypes.

4.11 Supplemental Information

Table 4.S1: Ranking criteria and model structures for candidate models describing total mercury concentrations for benthic female stickleback from Benka Lake, Alaska. All models are based on a sample size of 175 fish and include additive (+) and interaction (*) terms.

Model Structure[a]	k[b]	LogL[c]	AIC_C[d]	ΔAIC_C	w_i[f]
α+C:N+JD+JD^2+SL+TP	9	3.29	12.51	0.00	0.47
C:N+JD+ JD^2+SL+TP	8	1.07	14.73	2.22	0.15
α+JD+ JD^2+SL+TP	8	0.79	15.29	2.78	0.12
α+C:N+SL+TP	7	-1.00	16.67	4.16	0.06
α+C:N+JD+SL+TP	8	0.05	16.76	4.25	0.06
α+C:N+ JD^2+SL+TP	8	-0.11	17.10	4.59	0.05
C:N+JD+SL+TP	7	-1.57	17.82	5.31	0.03
C:N+SL+TP	6	-2.77	18.03	5.52	0.03
C:N+ JD^2+SL+TP	7	-1.74	18.15	5.64	0.03
JD+ JD^2+SL+TP	7	-2.80	20.28	7.77	0.01
α+C:N+JD+ JD^2+TP	8	-2.94	22.76	10.25	0.00
α+JD+ JD^2+TP	7	-4.30	23.26	10.75	0.00
α+C:N+TP	6	-7.96	28.43	15.92	0.00
α+C:N+JD+TP	7	-7.26	29.19	16.68	0.00
C:N+JD+ JD^2+TP	7	-7.38	29.42	16.91	0.00
α+C:N+ JD^2+TP	7	-7.41	29.50	16.99	0.00
JD+ JD^2+TP	6	-9.95	32.40	19.90	0.00
α+JD+SL+TP	7	-9.50	33.68	21.17	0.00
C:N+TP	5	-11.84	34.04	21.53	0.00
C:N+JD+TP	6	-10.98	34.45	21.94	0.00
α+ JD^2+SL+TP	7	-9.99	34.65	22.14	0.00
C:N+ JD^2+TP	6	-11.14	34.78	22.27	0.00
α+SL+TP	6	-12.34	37.19	24.68	0.00
JD+SL+TP	6	-13.90	40.29	27.78	0.00
α+JD+TP	6	-14.03	40.55	28.04	0.00
α+ JD^2+TP	6	-14.43	41.36	28.85	0.00
JD^2+SL+TP	6	-14.45	41.39	28.88	0.00
α+TP	5	-16.22	42.80	30.29	0.00
SL+TP	5	-17.32	44.99	32.48	0.00
JD+TP	5	-20.45	51.25	38.74	0.00
JD^2+TP	5	-20.91	52.18	39.67	0.00

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Model Structure[a]	k[b]	LogL[c]	AIC_C[d]	ΔAIC_C	w_i[f]
TP	4	-23.18	54.60	42.09	0.00
α+C:N+JD+ JD^2+SL	8	-46.38	109.62	97.11	0.00
α+C:N+SL	6	-48.66	109.82	97.32	0.00
α+C:N+ JD^2+SL	7	-48.61	111.90	99.39	0.00
α+C:N+JD+SL	7	-48.64	111.95	99.44	0.00
α+JD+ JD^2+SL	7	-51.06	116.79	104.29	0.00
α+C:N+JD+ JD^2	7	-52.96	120.59	108.08	0.00
α+JD+ JD^2	6	-55.73	123.96	111.45	0.00
α+C:N	5	-57.65	125.65	113.14	0.00
α+C:N+ JD^2	6	-57.40	127.31	114.80	0.00
α+C:N+JD	6	-57.49	127.48	114.97	0.00
α+SL	5	-61.42	133.20	120.69	0.00
α+JD+SL	6	-60.76	134.03	121.52	0.00
α+ JD^2+SL	6	-60.97	134.43	121.92	0.00
α	4	-67.03	142.29	129.78	0.00
α+JD	5	-66.69	143.74	131.23	0.00
α+ JD^2	5	-66.84	144.03	131.52	0.00
C:N+JD+ JD^2+SL	7	-68.02	150.71	138.20	0.00
JD+ JD^2+SL	6	-69.51	151.51	139.00	0.00
JD+ JD^2	5	-70.92	152.19	139.68	0.00
C:N+JD+ JD^2	6	-69.88	152.27	139.76	0.00
C:N+ JD^2+SL	6	-70.65	153.79	141.28	0.00
C:N+JD+SL	6	-70.89	154.27	141.76	0.00
C:N+SL	5	-72.56	155.48	142.97	0.00
C:N+ JD^2	5	-73.76	157.87	145.37	0.00
C:N+JD	5	-74.07	158.50	145.99	0.00
SL	4	-75.86	159.96	147.45	0.00
C:N	4	-75.94	160.12	147.61	0.00
JD^2+SL	5	-75.60	161.55	149.04	0.00
JD+SL	5	-75.70	161.76	149.25	0.00
Null	3	-78.59	163.32	150.81	0.00
JD^2	4	-78.23	164.70	152.19	0.00
JD	4	-78.36	164.96	152.45	0.00

[a] Sex = sex, Pop = population, α = percent reliance on benthic prey, JD = Julian day (date) and TP = trophic position; [b] The number of estimated parameters in the model including the intercept and variance; [c] Log-likelihood of the regression model; [d] Sample size corrected Akaike's Information Criterion; [e] The difference between the current model AIC_C and the AIC_C of the most parsimonious model; [f] Akaike's weight; the likelihood of the current model relative to others in the candidate set.

Table 4.S2: Ranking criteria and model structures for candidate models describing total mercury concentrations for benthic male stickleback from Benka Lake, Alaska. All models are based on a sample size of 163 fish and include additive (+) and interaction (*) terms.

Model Structure[a]	k[b]	LogL[c]	AIC_C[d]	ΔAIC_C	w_i[f]
α+C:N+JD+JD2+SL+TP	9	4.36	10.42	0.00	0.35
α+C:N+JD+JD2+TP	8	2.76	11.38	0.96	0.22
α+JD+JD2+SL+TP	8	2.51	11.88	1.46	0.17
α+JD+JD2+TP	7	0.91	12.88	2.46	0.10
C:N+JD+JD2+SL+TP	8	1.20	14.51	4.09	0.05
α+C:N+SL+TP	7	-0.36	15.41	4.99	0.03
C:N+JD+JD2+TP	7	-0.86	16.42	6.00	0.02
α+C:N+JD2+SL+TP	8	0.09	16.73	6.31	0.02
α+C:N+JD+SL+TP	8	-0.04	16.98	6.56	0.01
α+C:N+TP	6	-2.93	18.39	7.97	0.01
α+C:N+JD2+TP	7	-1.98	18.67	8.24	0.01
α+C:N+JD+TP	7	-2.18	19.06	8.64	0.00
C:N+SL+TP	6	-3.28	19.08	8.66	0.00
JD+JD2+SL+TP	7	-2.50	19.71	9.28	0.00
C:N+JD2+SL+TP	7	-2.76	20.22	9.80	0.00
C:N+JD+SL+TP	7	-2.89	20.48	10.06	0.00
JD+JD2+TP	6	-4.70	21.93	11.51	0.00
C:N+TP	5	-6.29	22.95	12.53	0.00
C:N+JD2+TP	6	-5.26	23.04	12.62	0.00
C:N+JD+TP	6	-5.46	23.44	13.02	0.00
α+SL+TP	6	-6.54	25.61	15.19	0.00
α+TP	5	-8.52	27.40	16.98	0.00
α+JD2+SL+TP	7	-6.53	27.77	17.34	0.00
α+JD+SL+TP	7	-6.54	27.79	17.37	0.00
α+JD2+TP	6	-8.38	29.27	18.85	0.00
α+JD+TP	6	-8.46	29.44	19.02	0.00
SL+TP	5	-12.09	34.54	24.12	0.00
JD2+SL+TP	6	-12.07	36.66	26.24	0.00
JD+SL+TP	6	-12.09	36.69	26.27	0.00
TP	4	-14.60	37.45	27.03	0.00
JD2+TP	5	-14.46	39.29	28.87	0.00
JD+TP	5	-14.54	39.45	29.03	0.00
α+C:N+JD+JD2	7	-24.91	64.52	54.10	0.00

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Model Structure[a]	k[b]	LogL[c]	AIC_C[d]	ΔAIC_C	w_i[f]
α+C:N+JD+JD2+SL	8	-24.51	65.93	55.51	0.00
α+C:N+JD2	6	-29.38	71.29	60.87	0.00
α+C:N+JD	6	-29.80	72.12	61.70	0.00
α+C:N+JD2+SL	7	-28.78	72.26	61.84	0.00
α+C:N+JD+SL	7	-29.17	73.04	62.62	0.00
C:N+JD+JD2	6	-30.72	73.96	63.54	0.00
α+JD+JD2	6	-31.17	74.86	64.44	0.00
α+C:N	5	-32.81	75.98	65.56	0.00
C:N+JD+JD2+SL	7	-30.72	76.13	65.71	0.00
α+JD+JD2+SL	7	-30.87	76.44	66.02	0.00
α+C:N+SL	6	-32.00	76.53	66.10	0.00
JD+JD2	5	-35.17	80.72	70.30	0.00
C:N+JD2	5	-35.44	81.25	70.83	0.00
C:N+JD	5	-35.98	82.33	71.90	0.00
JD+JD2+SL	6	-35.17	82.87	72.45	0.00
C:N+JD2+SL	6	-35.40	83.33	72.91	0.00
C:N+JD+SL	6	-35.94	84.40	73.98	0.00
C:N	4	-40.23	88.71	78.28	0.00
C:N+SL	5	-40.18	90.73	80.31	0.00
α+JD2	5	-41.10	92.57	82.15	0.00
α	4	-42.43	93.11	82.69	0.00
α+JD	5	-41.40	93.16	82.74	0.00
α+JD2+SL	6	-40.77	94.05	83.63	0.00
α+SL	5	-41.99	94.34	83.92	0.00
α+JD+SL	6	-41.05	94.62	84.20	0.00
JD2	4	-44.57	97.38	86.96	0.00
JD	4	-44.94	98.13	87.71	0.00
Null	3	-46.60	99.35	88.93	0.00
JD2+SL	5	-44.55	99.47	89.05	0.00
JD+SL	5	-44.92	100.21	89.79	0.00
SL	4	-46.57	101.38	90.96	0.00

[a] Sex = sex, Pop = population, α = percent reliance on benthic prey, JD = Julian day (date) and TP = trophic position; [b] The number of estimated parameters in the model including the intercept and variance; [c] Log-likelihood of the regression model; [d] Sample size corrected Akaike's Information Criterion; [e] The difference between the current model AIC_C and the AIC_C of the most parsimonious model; [f] Akaike's weight; the likelihood of the current model relative to others in the candidate set.

Table 4.S3: Ranking criteria and model structures for candidate models describing total mercury concentrations for limnetic female stickleback from Benka Lake, Alaska. All models are based on a sample size of 168 fish and include additive (+) and interaction (*) terms.

Model Structure[a]	k[b]	LogL[c]	AIC_C[d]	ΔAIC_C	w_i[f]
C:N+SL+TP	6	-5.74	24.03	0.00	0.27
C:N+JD+JD2+SL+TP	8	-3.70	24.36	0.33	0.23
C:N+JD+SL+TP	7	-5.40	25.53	1.51	0.13
C:N+JD2+SL+TP	7	-5.49	25.71	1.68	0.12
α+C:N+SL+TP	7	-5.71	26.15	2.13	0.09
α+C:N+JD+JD2+SL+TP	9	-3.69	26.57	2.55	0.08
α+C:N+JD+SL+TP	8	-5.39	27.72	3.69	0.04
α+C:N+JD2+SL+TP	8	-5.47	27.89	3.87	0.04
C:N+TP	5	-12.04	34.46	10.43	0.00
C:N+JD+JD2+TP	7	-10.16	35.05	11.03	0.00
C:N+JD2+TP	6	-12.03	36.61	12.58	0.00
α+C:N+TP	6	-12.03	36.61	12.59	0.00
C:N+JD+TP	6	-12.04	36.62	12.59	0.00
α+C:N+JD+JD2+TP	8	-10.14	37.22	13.19	0.00
α+C:N+SL	6	-12.77	38.09	14.06	0.00
α+C:N+JD2+TP	7	-12.03	38.79	14.76	0.00
α+C:N+JD+TP	7	-12.03	38.80	14.77	0.00
α+C:N+JD+JD2+SL	8	-11.26	39.46	15.43	0.00
α+C:N+JD2+SL	7	-12.67	40.07	16.04	0.00
α+C:N+JD+SL	7	-12.71	40.15	16.12	0.00
α+C:N+JD+JD2	7	-16.68	48.08	24.06	0.00
α+C:N	5	-18.90	48.18	24.16	0.00
α+C:N+JD2	6	-18.31	49.16	25.14	0.00
α+C:N+JD	6	-18.42	49.38	25.36	0.00
α+JD+JD2+SL+TP	8	-23.62	64.18	40.15	0.00
C:N+JD+JD2+SL	7	-26.25	67.22	43.20	0.00
JD+JD2+SL+TP	7	-27.81	70.35	46.32	0.00
α+SL+TP	6	-29.11	70.77	46.74	0.00
C:N+JD+JD2	6	-29.29	71.13	47.10	0.00
α+JD+SL+TP	7	-28.43	71.58	47.56	0.00
α+JD2+SL+TP	7	-28.60	71.94	47.91	0.00
C:N+JD2+SL	6	-30.07	72.68	48.66	0.00
C:N+SL	5	-31.32	73.02	49.00	0.00

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Model Structure[a]	k[b]	LogL[c]	AIC_C[d]	ΔAIC_C	w_i[f]
C:N+JD+SL	6	-30.30	73.15	49.12	0.00
α+JD+JD2+TP	7	-29.30	73.33	49.31	0.00
SL+TP	5	-32.32	75.02	51.00	0.00
JD+SL+TP	6	-31.83	76.20	52.18	0.00
JD2+SL+TP	6	-31.97	76.48	52.46	0.00
C:N+JD2	5	-33.21	76.82	52.79	0.00
C:N+JD	5	-33.49	77.37	53.34	0.00
C:N	4	-34.89	78.05	54.02	0.00
α+TP	5	-34.35	79.08	55.06	0.00
JD+JD2+TP	6	-33.71	79.97	55.95	0.00
α+JD+TP	6	-34.11	80.76	56.74	0.00
α+JD2+TP	6	-34.20	80.95	56.93	0.00
α+JD+JD2+SL	7	-33.66	82.05	58.02	0.00
JD+JD2+SL	6	-35.70	83.94	59.92	0.00
TP	4	-38.00	84.26	60.23	0.00
α+SL	5	-37.76	85.91	61.88	0.00
JD+TP	5	-37.87	86.12	62.10	0.00
JD2+TP	5	-37.93	86.26	62.23	0.00
α+JD+SL	6	-37.74	88.02	63.99	0.00
α+JD2+SL	6	-37.76	88.06	64.03	0.00
SL	4	-40.38	89.01	64.98	0.00
α+JD+JD2	6	-38.45	89.45	65.43	0.00
JD+JD2	5	-39.63	89.64	65.61	0.00
JD2+SL	5	-40.25	90.88	66.86	0.00
JD+SL	5	-40.31	91.01	66.99	0.00
α	4	-42.79	93.84	69.81	0.00
Null	3	-44.53	95.21	71.18	0.00
α+JD2	5	-42.74	95.87	71.84	0.00
α+JD	5	-42.78	95.94	71.92	0.00
JD2	4	-44.27	96.79	72.76	0.00
JD	4	-44.36	96.98	72.96	0.00

[a] Sex = sex, Pop = population, α = percent reliance on benthic prey, JD = Julian day (date) and TP = trophic position; [b] The number of estimated parameters in the model including the intercept and variance; [c] Log-likelihood of the regression model; [d] Sample size corrected Akaike's Information Criterion; [e] The difference between the current model AIC_C and the AIC_C of the most parsimonious model; [f] Akaike's weight; the likelihood of the current model relative to others in the candidate set.

Table 4.S4: Ranking criteria and model structures for candidate models describing total mercury concentrations for limnetic male stickleback from Benka Lake, Alaska. All models are based on a sample size of 161 fish and include additive (+) and interaction (*) terms.

Model Structure[a]	k[b]	LogL[c]	AIC_C[d]	ΔAIC_C	w_i[f]
α+C:N+JD+JD2+SL+TP	9	31.95	-44.71	0.00	0.67
C:N+JD+JD2+SL+TP	8	29.29	-41.64	3.07	0.14
α+JD+JD2+SL+TP	8	29.08	-41.22	3.50	0.12
α+C:N+JD2+SL+TP	8	27.30	-37.67	7.05	0.02
α+C:N+JD+SL+TP	8	27.02	-37.11	7.61	0.02
α+C:N+SL+TP	7	25.65	-36.57	8.14	0.01
C:N+JD2+SL+TP	7	25.01	-35.31	9.41	0.01
α+C:N+JD+JD2+TP	8	25.90	-34.86	9.85	0.00
C:N+JD+SL+TP	7	24.73	-34.74	9.98	0.00
C:N+SL+TP	6	23.22	-33.91	10.81	0.00
α+JD+JD2+TP	7	23.36	-32.00	12.72	0.00
C:N+JD+JD2+SL	7	22.85	-30.97	13.74	0.00
α+C:N+JD+JD2+SL	8	23.08	-29.23	15.48	0.00
C:N+JD+JD2+TP	7	21.80	-28.88	15.83	0.00
α+SL+TP	6	20.41	-28.28	16.44	0.00
α+JD2+SL+TP	7	21.07	-27.43	17.29	0.00
α+JD+SL+TP	7	20.88	-27.03	17.68	0.00
JD+JD2+SL+TP	7	20.57	-26.42	18.29	0.00
α+C:N+JD2+TP	7	20.48	-26.23	18.48	0.00
α+C:N+JD+TP	7	20.11	-25.50	19.22	0.00
α+C:N+TP	6	18.09	-23.65	21.06	0.00
C:N+JD+JD2	6	17.47	-22.39	22.32	0.00
C:N+JD2+SL	6	17.28	-22.02	22.69	0.00
JD+JD2+SL	6	17.15	-21.75	22.96	0.00
α+JD+JD2+SL	7	17.91	-21.10	23.62	0.00
C:N+JD2+TP	6	16.82	-21.09	23.62	0.00
C:N+JD+SL	6	16.81	-21.09	23.62	0.00
α+C:N+JD2+SL	7	17.84	-20.97	23.75	0.00
C:N+JD+TP	6	16.44	-20.34	24.38	0.00
α+C:N+JD+JD2	7	17.47	-20.22	24.50	0.00
α+C:N+JD+SL	7	17.41	-20.10	24.61	0.00
C:N+TP	5	14.17	-17.95	26.77	0.00
C:N+SL	5	13.83	-17.28	27.44	0.00

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Model Structure[a]	k[b]	LogL[c]	AIC_C[d]	ΔAIC_C	w_i[f]
α+C:N+SL	6	14.63	-16.72	27.99	0.00
α+JD2+TP	6	14.56	-16.58	28.14	0.00
α+TP	5	13.43	-16.48	28.23	0.00
α+JD+TP	6	14.29	-16.04	28.67	0.00
α+JD+JD2	6	12.78	-13.02	31.70	0.00
C:N+JD2	5	11.43	-12.47	32.24	0.00
JD+JD2+TP	6	12.35	-12.16	32.56	0.00
C:N+JD	5	10.91	-11.44	33.28	0.00
JD+JD2	5	10.61	-10.84	33.87	0.00
α+C:N+JD2	6	11.46	-10.39	34.33	0.00
SL+TP	5	10.22	-10.05	34.67	0.00
α+C:N+JD	6	10.95	-9.37	35.35	0.00
JD2+SL+TP	6	10.63	-8.72	36.00	0.00
JD+SL+TP	6	10.47	-8.41	36.31	0.00
C:N	4	7.52	-6.79	37.92	0.00
α+C:N	5	7.62	-4.85	39.87	0.00
α+JD2+SL	6	8.32	-4.10	40.62	0.00
JD2+SL	5	7.17	-3.96	40.75	0.00
SL	4	6.03	-3.80	40.91	0.00
α+JD+SL	6	7.99	-3.44	41.27	0.00
JD+SL	5	6.90	-3.42	41.29	0.00
α+SL	5	6.75	-3.11	41.60	0.00
α+JD2	5	2.37	5.63	50.35	0.00
α+JD	5	1.99	6.40	51.12	0.00
α	4	0.36	7.54	52.25	0.00
TP	4	0.26	7.74	52.45	0.00
JD2+TP	5	1.02	8.33	53.05	0.00
JD+TP	5	0.80	8.78	53.50	0.00
JD2	4	-0.61	9.48	54.20	0.00
Null	3	-1.92	9.98	54.70	0.00
JD	4	-0.91	10.07	54.79	0.00

[a] Sex = sex, Pop = population, α = percent reliance on benthic prey, JD = Julian day (date) and TP = trophic position; [b] The number of estimated parameters in the model including the intercept and variance; [c] Log-likelihood of the regression model; [d] Sample size corrected Akaike's Information Criterion; [e] The difference between the current model AIC_C and the AIC_C of the most parsimonious model; [f] Akaike's weight; the likelihood of the current model relative to others in the candidate set.

Chapter 5: General Conclusions

The three studies presented here utilize the ecological diversity displayed in the Cook Inlet adaptive radiation of threespine stickleback (*Gasterosteus aculeatus*) in order to examine the drivers of intra- and inter-population variation in mercury (Hg) concentrations. Collectively my results demonstrate that the roles of trophic factors, especially trophic position and habitat-specific foraging, are varied and often differ by date, sex, ecotype, and population. Thus, generalizations across these groups are not likely to provide reliable estimates of mercury bioaccumulation. This conclusion is of particular importance considering these generalizations pervade the literature, especially for prey fishes such as stickleback.

The role of ecological drivers in determining Hg concentrations in fishes has been a major avenue of research for decades [1-6]. Despite the wealth of research demonstrating complex interactions between ecological and physiological factors on Hg bioaccumulation [7-16], the ecodynamics of Hg in fishes are largely summarized as simple and well established relationships with age, size, and TP [15].

The substantial variation I observed in the roles of ecological factors in the determination of stickleback THg concentrations indicates that these processes are more complex than the bulk of literature would suggest, representing the confounding effects of multiple interactions at many levels. Thus, further research is necessary to determine the mechanisms underlying these complex interactions. That future research must account for the hierarchical nature of Hg biogeochemistry by examining individual level

factors, such as I have presented, within the broader context of variation in populations, communities, ecosystems, and landscapes [17]. A major challenge of these studies will be to account for the subtle differences that exist even among populations that are ecologically convergent. I feel that the repeated, parallel adaptive radiations observed in postglacial fishes provide an opportunity to overcome this challenge; however, a large number of populations must be studied in order to obtain sufficient statistical power to tease apart subtle and often confounding effects.

My results suggest that additional controlled experiments examining the roles of individual ecological and physiological factors are necessary. Stickleback are easily reared under a variety of laboratory conditions and thus would be amenable to captive studies examining the roles of environmental and physiological factors, their interactions, and the mechanisms by which these factors influence Hg accumulation in fish. These experiments would be of particular value for the identification of common mechanisms underlying multiple factors. Such experiments, coupled with data from wild populations, would significantly advance our understanding of Hg bioaccumulation in fishes and their predators.

Across the three studies presented here I found a strong influence of sex on Hg dynamics. Mercury concentrations and the factors determining them differed between females and males regardless of the scale examined (i.e., inter- versus intra-population); however, the magnitude and direction of these differences were highly variable. In Benka Lake, female stickleback of both ecotypes had lower Hg concentrations than the males of their respective ecotype. In contrast, among the allopatric populations I

examined in chapter 3 females had higher mean Hg concentrations in four of the six populations examined.

These patterns suggest that the differences between the sexes are not the result of simple first-order physiological differences. Rather, these differences either directly reflect variable ecologies or are determined by physiological and behavioral processes dependent on ecological differences. Indeed, my seasonal examination of Hg concentrations in Benka Lake demonstrated that when differences in trophic position, habitat-specific foraging, size, and body condition were accounted for, females and males of the benthic ecotype were no longer significantly different. In contrast, the difference between females and males of the limnetic ecotype increased. This divergence in the response of the two ecotypes underscores the complexity of ecological and physiological interactions. Laboratory studies utilizing fishes reared under common conditions are necessary to determine if such differences are the result of variable ecologies, genetically controlled phenotypic differentiation, or genotype-by-environment interactions. Such studies have been widely used to address evolutionary questions with stickleback [18] and would provide a foundation by which we could advance our understanding of the drivers of Hg accumulation.

I also found that trophic position was consistently important in determining THg concentrations in stickleback, though again, the relative importance varied among sexes, ecotypes, and populations. The importance of trophic position is expected considering the known biomagnification of Hg in aquatic food webs [15]; however, the current understanding of Hg biomagnification suggests that there should be a relatively

consistent relationship between THg concentrations and trophic position [19]. My results suggest that this may not be the case; though in the current studies the potential for confounding ecological effects are admittedly high. Thus, further research directed at isolating the effects of trophic position is necessary to determine the mechanisms underlying variation in the relationship between trophic position and Hg concentration.

My data suggest that habitat-specific processes are important in determining THg concentrations in stickleback, but their effects are difficult to interpret due to covariation with other factors. Specifically, my data suggest that differences between benthic foraging and limnetic foraging stickleback likely represent a balance between increased Hg bioavailability in benthic habitats and increases in trophic position associated with limnetic foraging. This conclusion is supported by the results of my comparisons across populations (Chapter 3) and my examination of temporal variation in Benka Lake (Chapter 4). When individuals from all populations were analyzed together, I found that reliance on benthic prey (α) was positively correlated with non-normalized THg concentrations. This result is in contrast to an extensive body of literature suggesting that Hg concentrations are higher in limnetic foraging individuals [8, 13, 20-26], but in agreement with higher bioavailability of Hg in benthic habitats [27-32]. When variation in the THg concentrations of primary consumers was accounted for, the relationship between α and THg concentrations in stickleback was no longer observed.

Similarly, my work with Benka Lake stickleback demonstrates that while initial analyses suggest a negative correlation between α and THg concentration, much of this relationship is due to correlated effects with other variables. Once all other variables

were accounted for, Benka Lake stickleback displayed a positive effect of α. These data suggest that differential bioavailability and concentration of Hg in benthic and limnetic habitats is a key component of habitat-specific differences in THg concentrations of fishes and underscores the need to examine these results in the context of ecosystem processes regulating Hg bioavailability.

Overall, the results of this dissertation demonstrate substantial variation in the importance of individual factors in determining THg concentrations of threespine stickleback. While this variation undermines our ability to fully understand the mechanisms regulating Hg accumulation in fishes, it also provides a valuable study system to explore the complexities inherent in the ecodynamics of Hg. As with most research, a greater understanding comes with the realization that our previous understanding was simplistic. Further, when coupled with controlled laboratory studies designed to isolate independent effects of ecological and physiological variables, the variability observed in wild stickleback populations may provide important insights into the processes regulating Hg transfer to higher trophic level consumers such as piscivorous fish, birds, and mammals. Ultimately, these results suggest that future research should progress beyond merely accounting for these factors to the examination of the mechanistic processes underlying their effects and interactions.

5.1 Literature Cited

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